ALLEN'S
COMMERCIAL ORGANIC ANALYSIS

FOURTH EDITION

IN many respects this edition of Allen will be a new work. The field of Commercial Organic Analysis has been so enlarged and specialised during the last few years that it was found necessary to rewrite many parts and add much new matter. Obsolete methods are omitted; what little of the old text remains has been carefully revised and many new illustrations added.

To accomplish the object in view, namely, the furnishing of a modern work of the greatest practical value to the analyst, it was deemed advisable to secure the services of an English and an American editor and to organise a corps of writers particularly versed in the subjects discussed. Aside from those who have written for this volume, the following gentlemen have promised to write or revise articles for the forthcoming ones: J. A. Gardner, Chemical Department, St. George's Hospital, London; A. Marshall, Chief Inspector of Explosives, Indian Army; A. H. Gill, Boston; F. C. Garrett, Armstrong College, New Castle-on-Tyne; Samuel S. Sadtler, Philadelphia; C. A. Mitchell, London; Leonard Archbutt, Derby; C. A. Klein, London; William Robertson, London; W. P. Dreaper, author of "The Chemistry and Physics of Dyeing"; Cecil Revis, London; Edward Horton, Chelsea; Dr. M. B. Blackler, Ilford; E. W. Lewis, Laughton; J. Merritt Matthews, formerly Professor of Chemistry and Dyeing, Philadelphia Textile School; Dr. T. M. Lowry, London; E. J. Parry, London; Charles H. LaWall, Assistant in Theory and Practice of Pharmacy, Philadelphia College of Pharmacy.

The general arrangement of the volumes remains as before, only such changes have been made as will bring the text into line with the latest scientific classification. Great care has been exercised by the editors and contributors in the choice of methods and only those of the highest degree of accuracy and rapidity selected. Effort has been made to secure uniformity in weights and measures, nomenclature and abbreviations. References are to original sources, not to translations or abstracts.

The work will be issued in eight volumes, numbered consecutively, and will be published as rapidly as possible. Volume II is in press; Volumes III and IV are about ready for press; the remainder will follow as quickly as is consistent with good work. Complete announcement of the contents of, and contributors to, each volume will be made at a later date.

THE PUBLISHERS.

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COMMERCIAL ORGANIC ANALYSIS

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ALLEN'S COMMERCIAL
ORGANIC ANALYSIS

A TREATISE ON

THE PROPERTIES, MODES OF ASSAYING, AND PROXIMATE
ANALYTICAL EXAMINATION OF THE VARIOUS
ORGANIC CHEMICALS AND PRODUCTS
EMPLOYED IN THE ARTS, MANU-
FACTURES, MEDICINE, Etc.

WITH CONCISE METHODS FOR

THE DETECTION AND ESTIMATION OF THEIR IMPURITIES,
ADULTERATIONS, AND PRODUCTS OF DECOMPOSITION

VOLUME I

Introduction, Alcohols, Yeast, Malt Liquors and Malt, Wines and Spirits, Neutral
Alcoholic Derivatives, Sugars, Starch and its Isomerides, Paper and
Paper-making Materials, Vegetable Acids

BY THE EDITORS AND THE FOLLOWING CONTRIBUTORS

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FOURTH EDITION. ENTIRELY REWRITTEN

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PREFACE TO FOURTH EDITION.

SINCE the publication of the third edition of this work, the subject matter of organic chemistry has been so much extended that, in issuing a new edition, it has been necessary to allot the revision to specialists, each entrusted with the task of bringing a particular section up to date. This is, indeed, the plan that the distinguished author of the work had in view during the latter years of his life when the question of revision was pressing upon him.

Under this arrangement, the work has been almost entirely rewritten so that practically only the general plan of the earlier editions has been retained. Much descriptive matter now fully treated in text-books on chemistry and technology has been omitted, the object being to limit the work to its specific field of "Commercial Organic Analysis." It has been necessary to make some changes in the distribution of topics. Examination of Malt has been transferred to the section on Malt Liquors. The subject of Cellulose Nitrates has been transferred to the section on Smokeless Explosives, being included in the revised Volume II. Special articles on Yeast and on Paper and Paper-making Materials have been added to the present volume. A uniform system of nomenclature and abbreviations has been established and will be followed throughout the work. The decimal system of weights and measures will be used, except when special conditions render other standards necessary. Unless otherwise stated, all temperatures are centigrade and all readings of scale and arc positive.

By the selection of contributors from both sides of the Atlantic, the work has been made more distinctly international and thus better adapted to its wide field of usefulness. The editors, appreciating deeply the honour of directing the revision of a work that since its appearance has been in the front rank of authorities in the chemical laboratory, have endeavoured to be worthy of the task and of the co-operation of the contributors, and hope that the revised edition will maintain the reputation of the work as the most comprehensive and most representative treatise on "Commercial Organic Analysis" in any language.

NOVEMBER, 1909.

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INTRODUCTION.

By WILLIAM A. DAVIS, B. Sc., A. C. G. I.

THE term Analysis, though originally meaning the separation or splitting up of a substance into its constituent parts, has now become greatly extended in its application, so that a process of chemical analysis may mean either

A true analysis, or separation of a substance into its constituent parts;

A qualitative identification or recognition of a substance sought for; or

A quantitative estimation made with more or less accuracy of the composition of a substance.

When the quantitative analysis is limited to one or two important substances which constitute the valuable or active constituents of a more complex material, the analytical process is frequently called an *assay*. It is in this sense the term assay is employed throughout this work.

Very frequently the chemical examination of a substance includes the search for, or estimation of, impurities and foreign constituents accidentally present or purposely added. The nature of the foreign ingredients will, of course, largely depend upon that of the substance, and cannot be generally described. They may, however, be conveniently classified under the following heads:

Foreign substances naturally associated with the main substance, and not readily removed during the process of preparation. *Examples:* acetone in wood spirit; hydrogen cyanide in bitter-almond oil, and cresylic acid in carbolic acid.

Foreign substances introduced during the process of manufacture, and not subsequently (perfectly) eliminated. *Examples:* potassium cyanate and carbonate in commercial cyanide; sulphuric acid and lead salts in organic acids; alcohol in ether.

Foreign substances legitimately added in small quantity, to confer some special property on the main substance. *Examples:* mineral acids in hydrocyanic acid; alcohol in chloroform.

I

Foreign substances produced by the spontaneous change of the main substance. *Examples:* benzoic acid in bitter-almond oil; metaldehyde in aldehyde; ethyl acetate in tincture of iron acetate.

Adulterants purposely added to increase the weight or bulk, to confer some special property, or to conceal weakness or inferiority of the main substance. *Examples:* water in spirituous and vinous liquids; tartaric acid in citric acid; nitrobenzene in bitter-almond oil.

In the physical and chemical examination of organic materials many methods are employed, the details of which will be given under the proper heads, but the following general principles are frequently employed for the recognition and quantitative examination of such substances.

A preliminary examination of the leading characters of the substance, such as its colour, taste, odour, microscopic appearance and crystalline form.

A determination of the relative density of the substance, sometimes in the solid form, more frequently in the liquid condition, and occasionally in the state of vapour. The density of the solution of a substance is often a character of value.

Observations and operations connected with a *change in the physical state* of the substance, such as determinations of its melting and boiling points, and its behaviour on distillation.

A study of the *optical properties* of the substance, including its refractive and dispersive powers, absorption-spectrum, fluorescence, and action on a ray of polarised light.

A determination of the ultimate or *elementary composition* of the body.

The behaviour of the substance with ordinary *solvents*.

The behaviour of the substance with other *reagents*.

An examination of the substance for *inorganic* impurities.

The foregoing methods of examination are chiefly applicable to the recognition of comparatively pure compounds, but the principles involved are continually applied in the practical proximate analysis and chemical examination of organic materials. Thus, from the behaviour of the associated substances, when examined by one or more of the above methods, a practical recognition, determination, or separation of the constituents of the sample is effected.

It is not proposed to describe the whole of the above methods of examination in detail, as many of them are processes with the general nature of which the user of this book is presumably acquainted. In

most cases the outline of the method of examination is alone indicated, but exceptions are made in cases in which the same methods are not in general use in the analysis of inorganic substances. Sufficient working details for the use of any one versed in simple chemical manipulation are given under the special articles devoted to the examination of the various organic preparations employed in commerce.

PRELIMINARY EXAMINATION.

When the organic subsance to be examined is of wholly unknown nature a judicious preliminary examination will often throw much light on its composition. The following points should not be lost sight of:

Colour.—The colours of organic bodies are not, as a rule, very characteristic, but there are some very remarkable exceptions. As a rule, blue vegetable colouring matters are rendered red by acids, and the blue colour is restored or changed to green by ammonia. Indigo is not affected. Vegetable yellows are generally turned brown by alkalies, and the colours restored by acids. The examination of the absorption-spectra of coloured organic substances often furnishes most valuable information (see page 33).

Taste.—This character must be observed with extreme caution, as many organic compounds are intensely poisonous. The safest way is to make a weak aqueous or alcoholic solution of the substance and taste a drop of the liquid cautiously. Acids are, as a rule, sour or astringent in taste. Alkaloids are usually bitter. The sugars and glycerin are sweet.

Odour.—The odour of organic compounds is often highly characteristic, and notably so in the case of the neutral alcoholic derivatives.

Microscopic Appearance.—In the case of solid bodies an examination under the microscope is often extremely useful. As a rule, the use of a high power is neither necessary nor desirable. The micro-polariscope affords a valuable means of identifying starches.

Crystalline Form.—This character is often of great service for the recognition of organic substances and especially as a test for purity. In the great majority of cases the crystals are too small or indistinct to admit of any goniometric determination, but the appearance of a substance under the microscope and especially its behaviour towards polarised light afford valuable evidence. Instances of the value of crystalline form as means of identification are to be found in the cases of

cholesterol, salicylic acid, tartaric acid, and some of the alkaloids and their salts.

Effect of Heat.—The behaviour of organic substances on heating is often highly characteristic. Solids should be heated in a small, dry test-tube. It is well to make an experiment first on a piece of platinum foil, as a few substances explode violently when heated. On ignition in the air all organic substances other than those conta ning metals are completely consumed. Sometimes volatilisation occurs without darkening; in other cases, a more or less voluminous residue of carbon is left, which is sometimes burned away only with great difficulty. Salts of organic acids containing metals of the alkalies or alkaline earths usually leave these metals as carbonates on being ignited in the air. Hence the presence of carbonate in the ash indicates the previous presence of an organic acid. Volatile heavy metals, such as arsenic or mercury, are wholly driven off on igniting substances containing them, but most heavy metals remain on ignition either as oxides or in the metallic state.

The specific gravity, boiling and melting points, and other physical properties of the substance may be roughly noted as part of the preliminary examination, but these characters are referred to at greater length in the following sections.

SPECIFIC GRAVITY OR RELATIVE DENSITY

The specific gravity of an organic solid or liquid is often a most valuable criterion of its identity or purity. Unlike the determination of the density of a vapour, it is frequently applicable to the accurate estimation of a substance in solution or in admixture with another body, and in other cases it m :y be used to discriminate between substances of the same percentage composition.

The relative density of a solid or liquid is generally referred to water taken either as *unity* or as 1000. Both plans have their advantages, and, as no confusion can arise from such a course, the sp. gr. given in this work will be stated in e ther manner, according to convenience of expression or comparison.

The specific gravity bottle is the most generally serviceable means of taking the sp. gr. of solids and liquids. It should not be trusted to contain the amount of water marked on it, but should be filled with distilled water at the temperature at which the sample of liquid is to

be compared, and the weight of contained water ascertained. The sp. gr. of the sample is found by dividing the weight of it which the bottle contains by the weight of water contained at the same temperature. When the liquid is miscible with water, the wet bottle may be rinsed out once or twice with a few drops of the sample; when the liquid is immiscible or nearly so with water, the bottle should be rinsed once or twice with alcohol and then with ether, the last traces of the latter being got rid of by a current of dry air from a bellows, or by sucking the ether-vapour from the warmed bottle by means of a glass tube.

The selection of the temperature of $15.5°$ ($60°$ F.) sometimes involves consid rable practical inconvenience especially in the summer months. Squibb has introduced a urinometer for $25°$ ($77°$ F.) which, in the ordinary use of this instrument, is a much more convenient temperature. The current United States Pharmacopœia has adopted this temperature. Squibb has devised a bottle which eliminates the inconvenience of operating at a special temperature. The annexed description is from *Ephemeris*, January, 1897.

The bottle (Fig. 1) should hold 100 grm. of recently-boiled distilled water at $20°$ at about 58 on a scale of 0 to 100. In weighing the water into the bottle, the fine adjustment to 0.001 grm. is made by use of narrow strips of blotting-paper that will pass easily down the bore of the graduated stem. When the 100 grms. are in the bottle, and the column stands between 50 and 65 divisions of the scale, the stopper is put in, a leaden ring is placed on the neck, and the whole immersed in a bath of broken ice and water until the column of water comes to rest. It should then read at zero of the scale, or not much above it, and the reading should be noted. If it reads below zero, the bottle is too large, and the stopper part of the stem must be ground farther into the bottle neck, until the reading, on new trial, brings the column a little above zero. The bottle is then put

FIG. 1.

into a bath at $25°$ and kept there, the bath being stirred,—until the column comes to rest, when it should read about 90 to 100 of the scale. Should it read above 100, while the lower limit is as far above the zero, the bottle is too small, and the end of the stopper must be ground off until the reading of the column is within the graduations at both ends of the scale.

With this bottle the sp. gr. can be taken at any of the temperatures
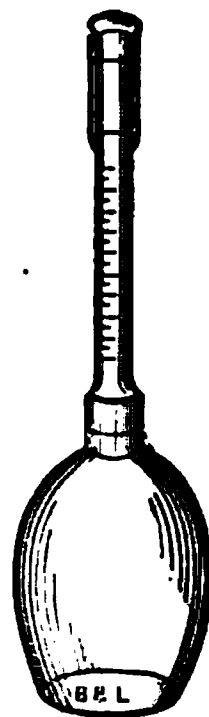
of the standard unit volume to the sixth decimal place, but the only way to avoid confusion is to state clearly the temperature at which the mass of the liquid and of water, respectively, were determined. Thus, for example, the sp. gr. of a substance expressed as 1.045 at 20°/4°, means that the value represents the ratio of the density of the substance at 20° to that of water at 4°. Compare on this point, Brown, Morris and Millar (*Trans.* 1897, **71**, 77, note). For the construction of a simple thermostat enabling the temperature to be kept within a few thousandths of a degree for long periods, see Lowry, *Trans.*, 1905, **87**, 1030, and *Trans. Faraday Soc.*, 1907, part iii. A description is given on p. 55.

Sprengel's Tube.—A useful method of taking the sp. gr. of liquids, especially when but small quantities are at disposal, is that of Sprengel

FIG. 2.   FIG. 3.

(*Jour. Chem. Soc.*, 1875, **26**, 577), in which a small U-shaped apparatus terminating in horizontal capillary tubes is substituted for the ordinary bottle. It may be easily filled and the regulation of the quanity of contained liquid is also easily effected. The results are of a high degree of accuracy. Sprengel's tube has the advantage that it can be used for ascertaining the sp. gr. at the b. p. of water. It consists (Fig. 2) essentially of a thin glass U-tube terminating in two capillary ends bent at right angles and each provided with a ground cap. One of

these capillary tubes must have a smaller calibre than the other—not larger than 0.25 mm. The larger tube should bear a mark at *m*. The tube is filled by immersing *b* in the liquid under examination, connecting the smaller end with a large glass bulb, and applying suction to the latter by means of a rubber tube, as shown in Fig. 3. If now the rubber tube be closed, the glass tube will fill automatically. It is placed in water, the ends being allowed to project, and the water is brought to the proper temperature. The Lowry thermostat may be used here with advantage. A conical flask may also be used to contain the water, the ends of the Sprengel tube being supported by the neck. The mouth of the flask should be loosely covered. As the liquid expands in the Sprengel tube it will drop from the larger orifice. When this ceases, the liquid is adjusted to the mark at *m*. If beyond the point, a little may be extracted by means of a roll of filter-paper. The tube is then taken out of the bath, the caps adjusted, the whole thoroughly dried, allowed to cool, and weighed. The same operation having been performed with distilled water, the calculation of the sp. gr. is made as usual.

For more elaborate directions as to use of this apparatus see Ostwald's *Physico-Chemical Measurements* or Findlay's *Practical Physical Chemistry*. For the rapid determination of the sp. gr. of liquids, especially saturated solutions, the Meyerhoffer-Saunders pipette, modified by Bousfield, is convenient. (See Lowry, *Trans.*, 1906, **89**, 1036.)

Hydrometers are instruments the use of which is too well known to require detailed description. Care should be taken in making accurate observations to read either from the top, bottom, or centre of the meniscus, according to the manner in which the instrument is graduated. Attention should also be paid to the temperature of the liquid during the observation.

The graduation of hydrometers, even when sold by well-known firms, is often far from accurate; hence the indications of such instruments should be carefully verified.

The accuracy of hydrometer-densities has been questioned in the case of milk and other liquids containing suspended particles, but the experiments of L. Siebold (*Analyst*, 1879, **4**, 189) show that the indications of the hydrometer in such cases agree with those obtained by the sp. gr. bottle.

Twaddell's hydrometer is applicable only to liquids heavier than water. The indications are translated into actual sp. gr. by mul-

tiplying the degrees Twaddell by 5 and adding 1000. Thus, a liquid which marks 68° Twaddell has an actual sp. gr. of $(5 \times 68) + 1000 = 1340$, compared with water as 1000.

Baumé's hydrometer is not commonly used in England, except for ascertaining the sp. gr. of saccharine solutions. As originally constructed, the point to which the instrument sank when immersed in a 10% solution by weight of common salt in water was taken as 10°. The interval between this point and that at which the hydrometer stood when immersed in pure water was divided into 10 equal parts, and a scale of similar equal parts extended as far as was necessary. Baudin (*Chem. News*, 1870, 54) found the sp. gr. of such a solution to be 1111 at 15°.

Much confusion and irregularity exist as to the scales of Baumé hydrometers commonly sold. C. F. Chandler (*Proc. National Acad. Sci.*, 1881, 3) found 36 different scales in use, many of them incorrect. According to Lunge (*Technical Methods of Chemical Analysis*, translated by Keane, vol. 1, part 1, 158 *et seq.*), the following formulæ are applicable for the conversion of Baumé degrees obtained by reference to a 10% salt solution (see below), into sp. gr., n representing the observed degree.

	Liquids heavier than water	Liquids lighter than water
At 12.5°	Sp. gr. $= \dfrac{145.88}{145.88 - n}$	Sp. gr. $= \dfrac{145.88}{135.88 + n}$
At 15.0°	Sp. gr. $= \dfrac{146.3}{146.3 - n}$	Sp. gr. $= \dfrac{146.3}{136.3 + n}$
At 17.5°	Sp. gr. $= \dfrac{146.78}{146.78 - n}$	Sp. gr. $= \dfrac{146.78}{136.78 + n}$

In a paper read before the New York Section of the Society of Chemical Industry (*J. Soc. Chem. Ind.*, 1905, 24, 781) the chemists of the laboratory of the (American) General Chemical Company use the following formula for converting Baumé degrees to sp. gr.

For liquids heavier than water: Sp. gr. $=\dfrac{145}{145-n}$ at 60° F.

For liquids lighter than water: Sp. gr. $=\dfrac{140}{130+n}$ at 60° F.

The so-called "rational" hydrometer, proposed originally by Kolb in France, but most widely used in Germany, is based on the following principle:

If a hydrometer sinks in water to the mark o°, and in a liquid D having a sp. gr. d to n°, then, as in each case the weight of the hydrometer W is equal to the weight of the liquid displaced, we have—

Wt. of the volume of water displaced by the hydrometer= W
Wt. of the same volume of liquid D $=d$W
Wt. of water displaced by n divisions of the scale $=n$
Wt. of same volume of liquid D $=dn$

For the weights dW and W to differ by nd,

$$d\text{W} - \text{W} = nd.$$
$$\therefore d = \frac{\text{W}}{\text{W}-n} \dots \dots (1)$$

Kolb calibrated his hydrometer by reference to "pure sulphuric acid of sp. gr. 1.842 at 15°." The point to which the hydrometer sank in the acid was indicated as 66° Bé.; with this method of calibration, from the above formula (1),

$$d = \frac{144.3}{144.3-n}$$

Although Kolb's system of calibration was based on an incorrect value for the sp. gr. of pure sulphuric acid (see Lunge, *op. cit.*), (the sp. gr. of 100% H_2SO_4 at 15°/4° being 1.8357), this method of calibration has been generally adopted in Germany, and was used for a time in the United States.

When the Baumé hydrometer is calibrated by reference to a 10% solution of pure sodium chloride (1 grm. in 9 grm. water) the following formula is obtained at 15°.

$$d = \frac{146.3}{146.3-n}$$

This method of calibration is known as that of Gerlach.

The Manufacturing Chemists Association of the United States of America in 1898 adopted (*J. Soc. Chem. Ind.*, 1898, **17**, 45) another method of calibration; in this scale "66° Bé." refers to sulphuric acid of

sp. gr. 1.835 at 15°/4° not because this is the highest obtainable strength, but because this is the sp. gr. of the acid sold and handled as "66° oil of vitriol" in commerce, which contains 93.5% of H_2SO_4 by weight.

TABLE I.

Comparison of Different Baumé Hydrometers with True Sp. Gr. *For Heavy Liquids.*

Degrees	Rational Scale $d=\dfrac{144.3}{144.3-n°}$ at 15°	Gerlach Scale $d=\dfrac{146.3}{146.3-n°}$ at 15°	American Scale $d=\dfrac{145}{145-n°}$ at 60° F.	M. C. A. at 15°/4°	Degrees	Rational Scale $d=\dfrac{144.3}{144.3-n°}$ at 15°	Gerlach Scale $d=\dfrac{146.3}{146.3-n°}$ at 15°	American Scale $d=\dfrac{145}{145-n°}$ at 60° F.	M. C. A. at 15°/4°
1	1.007	1.0068	1.007	1.005	34	1.308	1.3015	1.306	1.309
2	1.014	1.0138	1.014	1.011	35	1.320	1.3131	1.318	1.317
3	1.022	1.0208	1.021	1.023	36	1.332	1.3250	1.330	1.334
4	1.029	1.0280	1.028	1.029	37	1.345	1.3370	1.343	1.342
5	1.037	1.0353	1.036	1.036	38	1.357	1.3494	1.355	1.359
6	1.045	1.0426	1.043	1.043	39	1.370	1.3619	1.368	1.368
7	1.052	1.0501	1.051	1.050	40	1.383	1.3746	1.381	1.386
8	1.060	1.0576	1.058	1.057	41	1.397	1.3876	1.394	1.395
9	1.067	1.0653	1.066	1.064	42	1.410	1.4009	1.408	1.413
10	1.075	1.0731	1.074	1.071	43	1.424	1.4134	1.422	1.422
11	1.083	1.0810	1.082	1.086	44	1.438	1.4281	1.436	1.441
12	1.091	1.0890	1.090	1.093	45	1.453	1.4421	1.450	1.451
13	1.100	1.0972	1.098	1.100	46	1.468	1.4564	1.465	1.470
14	1.108	1.1054	1.107	1.107	47	1.483	1.4710	1.480	1.480
15	1.116	1.1138	1.115	1.114	48	1.498	1.4860	1.495	1.500
16	1.125	1.1224	1.124	1.122	49	1.514	1.5012	1.510	1.510
17	1.134	1.1310	1.133	1.136	50	1.530	1.5167	1.526	1.531
18	1.142	1.1398	1.142	1.143	51	1.546	1.5325	1.543	1.541
19	1.152	1.1487	1.151	1.150	52	1.563	1.5487	1.559	1.561
20	1.162	1.1578	1.160	1.158	53	1.580	1.5652	1.576	1.573
21	1.171	1.1670	1.169	1.172	54	1.597	1.5820	1.593	1.594
22	1.180	1.1763	1.179	1.179	55	1.615	1.5993	1.611	1.616
23	1.190	1.1858	1.188	1.186	56	1.634	1.6169	1.629	1.627
24	1.200	1.1955	1.198	1.201	57	1.652	1.6349	1.648	1.650
25	1.210	1.2053	1.208	1.208	58	1.671	1.6533	1.667	1.661
26	1.220	1.2153	1.218	1.216	59	1.691	1.6721	1.686	1.683
27	1.231	1.2254	1.229	1.231	60	1.711	1.6914	1.706	1.705
28	1.241	1.2357	1.239	1.238	61	1.732	1.7111	1.726	1.727
29	1.252	1.2462	1.250	1.254	62	1.753	1.7313	1.747	1.747
30	1.263	1.2569	1.261	1.262	63	1.774	1.7520	1.768	1.767
31	1.274	1.2677	1.272	1.269	64	1.796	1.7731	1.790	1.793
32	1.285	1.2788	1.283	1.285	65	1.819	1.7948	1.812	1.814
33	1.297	1.2901	1.295	1.293	66	1.842	1.8171	1.835	1.835

American Scales.

1. $\qquad$ Sp. gr. $= \dfrac{145}{145-n°}$ at 60° F.

See tables calculated by Emery, *J. Amer. Chem. Soc.*, 1899, **21**, 117.

2. Manufacturing Chemists Association of the U. S. A., *J. Soc. Chem. Ind.*, 1898, **17**, 45.[1]

The table on page 10 gives a comparison of the different scales with true sp. gr. (at 15°) for liquids heavier than water. As Göckel has pointed out (*Zeit. angew. Chem.*, 1903, 562), Baumé hydrometers should have inscribed on them not only the temperature for which they are calibrated, but also the temperature of the water used for comparison; it should also be stated whether the weights are referred to normal pressure (760 mm.) or to vacuum. In Germany, the Imperial Commission of Normal Standards (Kais. Normal Eichungs-Kommission, 1904, Heft 5) has recently thoroughly investigated the relation of degrees Baumé ("rational" scale) to true specific gravity. They give the following table (Table II.) for the transformation of sp. gr. at 15°/4° into degrees. Bé. (rational).

[1] The scale adopted by the (American) Manufacturing Chemists Association is a most irrational one as the values of sp. gr. plotted against degrees Baumé do not give a properly continuous curve. The non-scientific character of this scale is at once visible on considering the differences of sp. gr. for successive degrees Baumé; *e. g.*:

Degrees 1–2	Δ in sp. gr.	= 0.006	Degrees 10–11	Δ = 0.015
2–3	Δ in sp. gr.	= 0.012	11–12	Δ = 0.007
3–4	Δ in sp. gr.	= 0.006	12–13	Δ = 0.007
4–5	Δ in sp. gr.	= 0.007		

Degrees 19–20	Δ = 0.008
20–21	Δ = 0.014
21–22	Δ = 0.007

and so on.

In the upper part of the scale the differences are still more peculiar; *e. g.*:

Degrees 45–46	Δ = 0.019
46–47	Δ = 0.010
47–48	Δ = 0.020
Degrees 54–55	Δ = 0.022
55–56	Δ = 0.011
56–57	Δ = 0.011
58–59	Δ = 0.022

It would appear from the statement in the *J. Soc. Chem. Ind.*, 1905, 24, 782 that the M. C. A. has now adopted the scale of the (American) General Chemical Company (see page 8).

TABLE II.

Transformation of Sp. Gr. at 15°/4° into Degrees Baumé of the Rational Scale.

S 15/4	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0.99										−0.018
1.00	0.126	0.270	0.414	0.557	0.700	0.843	0.986	1.128	1.270	1.412
01	1.553	1.694	1.835	1.976	2.117	2.257	2.397	2.536	2.675	2.814
02	2.953	3.091	3.229	3.367	3.505	3.643	3.780	3.917	4.053	4.189
03	4.325	4.461	4.596	4.731	4.866	5.001	5.135	5.269	5.403	5.537
04	5.671	5.804	5.937	6.070	6.202	6.334	6.466	6.598	6.729	6.860
1.05	6.991	7.122	7.252	7.382	7.512	7.642	7.771	7.900	8.029	8.158
06	8.287	8.415	8.543	8.671	8.798	8.925	9.052	9.179	9.306	9.432
07	9.558	9.684	9.809	9.934	10.059	10.184	10.309	10.433	10.557	10.681
08	10.805	10.929	11.052	11.175	11.298	11.421	11.543	11.665	11.787	11.909
09	12.030	12.151	12.272	12.393	12.514	12.634	12.754	12.874	12.994	13.114
1.10	13.233	13.352	13.471	13.590	13.708	13.826	13.944	14.062	14.179	14.296
11	14.413	14.530	14.647	14.764	14.880	14.996	15.112	15.228	15.343	15.458
12	15.573	15.688	15.803	15.917	16.031	16.145	16.259	16.373	16.486	16.599
13	16.712	16.825	16.938	17.050	17.162	17.274	17.386	17.498	17.610	17.721
14	17.832	17.943	18.054	18.164	18.274	18.384	18.494	18.604	18.713	18.822
1.15	18.931	19.040	19.149	19.258	19.366	19.474	19.582	19.690	19.798	19.905
16	20.012	20.119	20.226	20.333	20.439	20.545	20.651	20.757	20.863	20.969
17	21.074	21.179	21.284	21.389	21.494	21.599	21.703	21.807	21.911	22.015
18	22.119	22.222	22.325	22.428	22.531	22.634	22.737	22.839	22.941	23.043
19	23.145	23.247	23.349	23.450	23.551	23.652	23.753	23.854	23.955	24.055
1.20	24.155	24.255	24.355	24.455	24.554	24.653	24.752	24.851	24.950	25.049
21	25.148	25.246	25.344	25.442	25.540	25.638	25.736	25.834	25.931	26.028
22	26.125	26.222	26.319	26.415	26.511	26.607	26.703	26.799	26.895	26.990
23	27.085	27.180	27.275	27.370	27.465	27.560	27.655	27.749	27.843	27.937
24	28.031	28.125	28.219	28.312	28.405	28.498	28.591	28.684	28.777	28.869
1.25	28.961	29.053	29.145	29.237	29.329	29.420	29.512	29.603	29.694	29.785
26	29.876	29.967	30.058	30.149	30.239	30.329	30.419	30.509	30.599	30.688
27	30.777	30.866	30.955	31.044	31.133	31.222	31.311	31.400	31.488	31.576
28	31.664	31.752	31.840	31.928	32.015	32.102	32.180	32.276	32.363	32.450
29	32.537	32.624	32.711	32.797	32.883	32.969	33.055	33.141	33.227	33.312
1.30	33.397	33.482	33.567	33.652	33.737	33.822	33.907	33.991	34.075	34.159
31	34.243	34.327	34.411	34.495	34.579	34.662	34.745	34.828	34.911	34.994
32	35.077	35.160	35.243	35.325	35.407	35.489	35.571	35.653	35.735	35.817
33	35.899	35.981	36.062	36.143	36.224	36.305	36.386	36.467	36.548	36.628
34	36.708	36.788	36.868	36.948	37.028	37.107	37.187	37.267	37.346	37.425
1.35	37.504	37.583	37.662	37.741	37.820	37.898	37.977	38.056	38.134	38.212
36	38.290	38.368	38.446	38.524	38.601	38.678	38.755	38.832	38.909	38.986
37	39.063	39.140	39.217	39.294	39.370	39.446	39.522	39.598	39.674	39.750
38	39.826	39.902	39.978	40.053	40.128	40.203	40.278	40.353	40.428	40.503
39	40.578	40.653	40.727	40.801	40.875	40.949	41.023	41.097	41.171	41.245
1.40	41.318	41.392	41.466	41.539	41.612	41.685	41.758	41.831	41.904	41.977
41	42.049	42.122	42.194	42.266	42.338	42.410	42.482	42.554	42.626	42.698

Transformation of Sp. Gr. at 15°/4° into Degrees Baumé of the Rational Scale.—*Con*.

S 15/4	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
1.42	42.769	42.840	42.912	42.983	43.054	43.125	43.196	43.267	43.338	43.409
43	43.479	43.550	43.620	43.690	43.760	43.830	43.900	43.970	44.040	44.110
44	44.179	44.248	44.318	44.387	44.456	44.525	44.594	44.663	44.732	44.801
1.45	44.869	44.938	45.007	45.075	45.143	45.211	45.279	45.347	45.415	45.483
46	45.551	45.619	45.687	45.754	45.821	45.888	45.955	46.022	46.089	46.156
47	46.223	46.290	46.357	46.423	46.489	46.555	46.621	46.687	46.753	46.819
48	46.885	46.951	47.017	47.083	47.148	47.213	47.279	47.344	47.409	47.474
49	47.539	47.604	47.669	47.734	47.799	47.863	47.928	47.992	48.056	48 1.0
1.50	48.184	48.248	48.312	48.376	48.440	48.503	48.567	48.631	48.694	48.757
51	48.820	48.884	48.947	49.010	49.073	49.136	49.199	49.262	49.325	49.387
52	49.449	49.512	49.574	49.636	49.698	49.760	49.822	49.884	49.946	50.008
53	50.069	50.131	50.193	50.254	50.315	50.376	50.437	50.498	50.559	50.620
54	50.681	50.742	50.803	50.864	50.924	50.984	51.045	51.105	51.165	51.225
1.55	51.285	51.345	51.405	51.465	51.525	51.584	51.643	51.703	51.763	51.822
56	51.881	51.940	51.999	52.058	52.117	52.176	52.235	52.294	52.353	52.411
57	52.469	52.528	52.587	52.645	52.703	52.761	52.819	52.877	52.935	52.993
58	53.051	53.109	53.167	53.225	53.282	53.339	53.397	53.454	53.511	53.568
59	53.625	53.682	53.739	53.796	53.853	53.909	53.966	54.023	54.079	54.135
1.60	54.191	54.248	54.304	54.360	54.416	54.472	54.528	54.584	54.640	54.696
61	54.751	54.807	54.863	54.918	54.973	55.028	55.083	55.138	55.193	55.248
62	55.303	55.358	55.413	55.468	55.523	55.577	55.632	55.687	55.742	55.796
63	55.850	55.904	55.958	56.012	56.066	56.120	56.174	56.228	56.282	56.336
64	56.389	56.443	56.497	56.550	56.603	56.656	56.709	56.763	56.816	56.869
1.65	56.922	56.975	57.028	57.081	57.134	57.186	57.239	57.292	57.344	57.396
66	57.448	57.501	57.553	57.605	57.657	57.709	57.761	57.813	57.865	57.917
67	57.968	58.020	58.072	58.124	58.175	58.226	58.278	58.329	58.380	58.431
68	58.482	58.533	58.584	58.635	58.686	58.737	58.788	58.839	58.890	58.940
69	58.990	59.041	59.092	59.142	59.192	59.242	59.292	59.342	59.392	59.442
1.70	59.492	59.542	59.592	59.641	59.691	59.741	59.791	59.840	59.890	59.939
71	59.988	60.038	60.087	60.136	60.185	60.234	60.283	60.332	60.381	60.430
72	60.478	60.527	60.576	60.625	60.673	60.721	60.770	60.818	60.866	60.914
73	60.962	61.010	61.058	61.106	61.154	61.202	61.250	61.298	61.346	61.394
74	61.441	61.489	61.537	61.585	61.632	61.679	61.727	61.774	61.821	61.868
1.75	61.915	61.962	62.009	62.056	62.103	62.150	62.197	62.244	62.291	62.337
76	62.383	62.430	62.477	62.523	62.569	62.615	62.662	62.708	62.754	62.800
77	62.846	62.892	62.938	62.984	63.030	63.075	63.121	63.167	63.213	63.258
78	63.303	63.349	63.395	63.440	63.485	63.530	63.576	63.621	63.666	63.711
79	63.756	63.801	63.846	63.891	63.936	63.980	64.025	64.070	64.115	64.159
1.80	64.203	64.248	64.293	64.337	64.381	64.425	64.469	64.514	64.558	64.602
81	64.646	64.690	64.734	64.778	64.822	64.866	64.910	64.954	64.998	65.041
82	65.084	65.128	65.172	65.215	65.258	65.301	65.345	65.388	65.431	65.474
83	65.517	65.560	65.603	65.646	65.689	65.731	65.774	65.817	65.860	65.902
84	65.944	65.987	66.030	66.073	66.115	66.157	66.200	66.242	66.284	66.326
1.85	66.368									

Great care must be exercised in expressing or interpreting results in the Baumé scale as, owing to the many different.systems in use, confusion may easily arise.

The values of sp. gr. corresponding with degrees Baumé in the tables given by the United States Department of Agriculture, Bulletin No 65 (1902), Table VI, and Bulletin No. 107 (1907), are apparently degrees of the rational scale,

$$d = \frac{144.3}{144.3 - n}$$

TABLE III.

Comparison of Degrees of Baumé Hydrometer for Light Liquids with Sp. Gr.

Degrees Baumé	Sp. gr. $= \frac{140}{130+n}$ at 60° F.	Sp. gr. $= \frac{146}{136+n}$ at 12.5°	Degrees Baumé	Sp. gr. $= \frac{140}{130+n}$ at 60° F.	Sp. gr. $= \frac{146}{136+n}$ at 12.5°	Degrees Baumé	Sp. gr. $= \frac{140}{130+n}$ at 60° F.	Sp. gr. $= \frac{146}{136+n}$ at 12.5°
10	1.0000	1.0000	27	0.8917	0.8957	44	0.8046	0.8111
11	0.9929	0.9932	28	0.8861	0.8902	45	0.8000	0.8066
12	0.9859	0.9865	29	0.8805	0.8848	46	0.7955	0.8022
13	0.9790	0.9799	30	0.8750	0.8795	47	0.7910	0.7978
14	0.9722	0.9733	31	0.8696	0.8742	48	0.7865	0.7935
15	0.9655	0.9669	32	0.8642	0.8690	49	0.7821	0.7892
16	0.9589	0.9605	33	0.8589	0.8639	50	0.7778	0.7849
17	0.9524	0.9542	34	0.8537	0.8588	51	0.7735	0.7807
18	0.9459	0.9480	35	0.8485	0.8538	52	0.7692	0.7766
19	0.9396	0.9420	36	0.8434	0.8488	53	0.7650	0.7725
20	0.9333	0.9359	37	0.8383	0.8439	54	0.7609	0.7684
21	0.9272	0.9299	38	0.8333	0.8391	55	0.7568	0.7643
22	0.9211	0.9241	39	0.8284	0.8343	56	0.7527	0.7604
23	0.9150	0.9183	40	0.8235	0.8295	57	0.7487	0.7565
24	0.9091	0.9125	41	0.8187	0.8249	58	0.7447	0.7526
25	0.9032	0.9068	42	0.8140	0.8202	59	0.7407	0.7487
26	0.8974	0.9012	43	0.8092	0.8156	60	0.7368	0.7449

Baumé hydrometers for liquids lighter than water are graduated in two ways:

1. The point to which the spindle sinks in a solution of 1 grm. of common salt in 9 grm. of water at 12.5° is called 0° and the point corresponding with pure water is called 10°. The degrees so obtained are repeated throughout the scale. This graduation gives:

$$\text{Sp. gr.} = \frac{145.88}{135.88 + n°}; \text{ or approximately}$$

$$\text{Sp. gr.} = \frac{146}{136 + n°}$$

2. In America (see tables given by Emery, *loc. cit.*) the divisions of the scale are obtained from the formula:

$$\text{Sp. gr.} = \frac{140}{130 + n^\circ} \text{ at } 60^\circ \text{ F.}$$

The table given on page 14 summarises the results of both methods of graduation.

Cartier's Hydrometer.—On this, 22° corresponds with 22° Baumé, but above and below this point the degrees are diminished in the ratio of 16 to 15.

Beck's Hydrometer.—The zero point corresponds to the sp. gr. of water and 30° to sp. gr. 850, the scale being divided into equal parts above and below the zero point, as far as desirable.

Other hydrometers are described in the section on *sugars*.

Unfortunately, much confusion has crept into the mode of stating sp. gr. Thus, if a liquid be stated to have a sp. gr. 0.7185 at 17.5°, there is no certainty as to what is intended. It may be meant that a bottle which holds 100 grm. of water at 17.5° holds only 71.85 grm. of the liquid, or the bottle may hold 100 grm. of water at 15.5° (60° F.), at 15.0°, at 4°, or at 0°. In many instances it is uncertain whether the recorded sp. gr. refers to a comparison with an equal volume of water at the same temperature as that at which the liquid was weighed or at any one of the temperatures just given. As a rule, when the sp. gr. of a substance is stated to have a given value at 15.5° (60° F.), it may be regarded as probable that the unit of water was weighed at the same temperature, but in the other cases it is not certain what is meant.

The sp. gr. of *organic solids* is best taken by introducing some fragments or powder into a sp. gr. bottle and ascertaining the weight. The bottle is next filled with water, petroleum, or some liquid of known density having no solvent action on the solid to be examined, and the weight is then again observed. The increase gives the weight of contained liquid, which divided by its known sp. gr., gives its volume. This subtracted from the known capacity of the bottle gives the volume of the solid, which, divided into its weight, gives the sp. gr. compared with water as unity. Care must be taken to avoid the adherence of air-bubbles to the solid. Agitation will generally suffice to remove them.

In many cases the Blount or Schumann bottle used in the examination of cement may be with advantage employed with a suitable solvent.

Hager has described (*Analyst*, 1876, 4, 206), a method of ascertaining

the sp. gr. of fats and similar bodies, by diluting alcohol or strong ammonia with water until suspended fragments of the substance remain in equilibrium in any part of the liquid at the standard temperature. The sp. gr. of the liquid is then taken, being the same as that of the solid.   This is an adaptation of the well known method used in ascertaining the sp. gr. of minerals.

Vapour-densities.—The determination of the vapour-density of an organic substance often furnishes confirmation of its formula.   In all cases in which decomposition of the substance does not occur, the density of the vapour, compared with that of hydrogen at the same temperature and pressure, is one-half the molecular weight.

The vapour-density of a volatile liquid is most rapidly ascertained by means of the method devised by Victor Meyer.   The molecular weight of a non-volatile substance can be ascertained by measuring the rise of b. p. or depression of the freezing point of a suitable solvent in which a known amount of the substance is dissolved.   For details of these methods see any treatise on practical physical chemistry; for example, Ostwald and Luther's *Physico-chemical Measurements* or Findlay's *Practical Physical.Chemistry*.

OBSERVATIONS OF CHANGES OF PHYSICAL STATE.

The **melting point** of an organic substance is best ascertained by heating a little of the substance in a capillary tube sealed at one end and about three inches long and 0.01 to 0.02 in diameter; such tubes are readily made by drawing out a test-tube in a blow-pipe flame.   The tube containing the substance is placed at the side of the bulb of a thermometer, so as to adhere to it, and heated in a small beaker of strong sulphuric acid, the temperature of which is gradually raised by means of a small burner placed beneath.   In order to make the reading of the m. p. as sharp as possible the temperature is raised only very slowly just before the substance melts.   After melting, the substance should be allowed to solidify and the m. p. again taken.   It must be borne in mind that although a pure substance generally melts quite sharply at a definite temperature (within 0.5°) the m. p. is much lowered and rendered indefinite by the presence of a small quantity of impurity. *The m. p. is only of value, therefore, in characterising a substance which has been carefully purified.*

The **subliming point** of an organic body is sometimes an important

characteristic, but its value depends much on the manner of making the observation. A. Wynter Blyth recommends the following method: A porcelain crucible about 3 ins. in diameter is nearly filled with mercury (or, for high temperatures, fusible metal). A minute quantity of the substance to be examined is placed on a thin disc of microscopic covering glass, which is floated on the mercury, and covered with a glass ring (cut from tubing), on which is placed a second disc so as to form a closed shallow cell. The porcelain crucible is placed on a brass plate and covered with a flask from which the bottom has been removed. This serves to keep away currents of air and supports the thermometer, which passes through a cork in the neck, so that the bulb is immersed in the mercury. In the first examination of a substance the temperature is raised somewhat rapidly, the upper disc being removed by forceps and exchanged for a fresh disc at every rise of 20°, until the substance is destroyed. A second determination is conducted more slowly and the discs more frequently changed, while in conducting the third determination the heat is raised very cautiously, and the discs changed every half degree when the previously ascertained subliming point is nearly reached. Blyth defines the subliming point as the lowest temperature, which, if maintained for 60 seconds, allows of the formation of the most minute dots, films, or crystals which can be observed by a microscopic power of 1/4 in.

The great majority of subliming points given in this work have not been determined in the above exact manner.

Boiling Point.—In making this determination care must be taken that the thermometer bulb is slightly above the surface of the liquid, which should be caused to boil rapidly. The liquid may be contained in a simple test-tube fitted with a cork carrying the thermometer and a short open tube for the escape of the vapour. A small tubulated flask or retort may be substituted for the test-tube. When the quantity of the liquid at disposal is only small, the test-tube should be thin and immersed in a flask half filled with glycerol, paraffin, sulphuric acid, or other suitable liquid. On heating the contents of the flask, the thermometer fitted to the test-tube continues to rise till the b. p. of the liquid is attained, when it remains stationary till the latter has evaporated. A very small quantity of liquid suffices for the determination of the b. p. in this manner.

For general purposes the apparatus of Berthelot is convenient. Fig. 4, from Traube's *Physico-chemical Methods*, shows its con-

struction.   The thermometer is enclosed in an outer tube, so that the portion of the scale to which the mercury rises is immersed in the vapour. If this be not done, a correction must be applied for the error produced by the cooling of the thermometer tube.   The bulb of the thermometer does not reach into the liquid.   A few fragments of pumice-stone or broken clay pipestem will prevent bumping.   The exit-tube at the lower end of the wide tube connects with a condenser.   The barometric pressure must always be noted and correction made for the departure from the standard pressure, 760 mm., by the following formula:

$$B = B^1 + 0.0375 (760 - P); \text{ in which}$$

B is the b. p. at normal pressure,
B^1 the observed b. p.,
P the observed pressure in mm.

Distillation does not need detailed description.   For cooling the vapour some form of Liebig's condenser is commonly employed.   A useful modification, by which distillation can be made at once to succeed digestion, without rearrangement of the apparatus, has been described by W. A. Shenstone, (*Trans.*, 1888, **53**, 123).

Fig. 4.

Recently several types of double surface condensers have been devised with the object of rendering the condensing action more efficient, so that shorter condensers may be employed than is possible with the old Liebig type.   The Cribb condenser is an instance of such a form.   Probably the most convenient for all-round work is Davies' condenser, made by Messrs. Gallenkamp, of London.

Fractional distillation is an analytical process closely related to the determination of the boiling and subliming points of organic substances; by repeating the process of distillation and collecting apart the fractions which distil at every small increase of temperature, very perfect separation may sometimes be effected.

When only a small quantity of a complex liquid is submitted to fractional distillation, it is better to keep the bulb of the thermometer wholly immersed in the liquid, as the error liable to be caused by this arrangement is far less than ensues, especially towards the end of the distillation, from the temperature of the residual liquid rising more

rapidly than the thermometer can acquire the temperature of the vapour.

In conducting a fractional distillation, it is desirable to operate on a known weight or volume of the substance, and to note the proportion of the whole which passes over at every few degress of rise in the temperature of the distilling liquid. Details of the precautions which should be taken to ensure constant results will be found in the section treating of the assay of commercial benzols.

Fractional distillation is a process of the utmost value for effecting the proximate analysis of a mixture of organic substances of different b. p. Speaking generally, the first portions which distil will contain the greater part of the more volatile constituents of a complex fluid, but the composition of the distillate at various stages of the process depends on many circumstances besides the b. p. and relative proportions of the constituents of the mixture operated upon.

Wanklyn showed that the proportion in which the constituents of a mixture pass over depends not only on their relative abundance in the mixture undergoing distillation, and on their respective vapour-tensions at the temperature of ebullition, but also on their mutual adhesion and on the densities of their vapours. He found that, when a mixture of equal weights of two liquids of different b. p. was distilled, the quantity of each constituent in the distillate was proportional to the product of its vapour-density and vapour-tension at the temperature of ebullition of the fraction. Hence, in certain cases, the less volatile of two substances may pass over most rapidly—that is, be found in largest quantity in the first fraction of the distillate. This is true of a mixture of methyl alcohol (boiling at $65.2°$) and ethyl iodide (boiling at $72°$). If the vapour-tensions and vapour-densities, of the two liquids are inversely proportional, the mixture will distil unchanged.

M. C. Lea found that, on distilling a mixture of ethylamine, diethylamine, and triethylamine hydrochlorides with sodium hydroxide, the whole of the last amine, which is the least volatile of the three, was contained in the first portions of the distillate, provided that its proportion was not excessive. A similar anomaly is observed on distilling solutions of acetic acid and its homologues.

Sometimes anomalous results ensue, owing to the fact that the tension of the mixed vapours is never equal to the sum of the tensions of the individual vapours. Berthelot found that when a mixture of 90.9 parts of carbon disulphide (boiling at $46.6°$), with 9.1 of alcohol (boiling

at 98.4°), was distilled, it behaved as a homogeneous liquid. If either of the constituents was present in excess of the above proportion, it remained in the retort in an unmixed condition after the definite mixture had distilled over. Thorpe, again, found that a mixture of equal volumes of methyl alcohol and carbon tetrachloride distilled at a temperature nearly 10° lower than that of the b. p. of the most volatile constituent, and the carbon tetrachloride, which has the higher b. p., occurred most largely in the first fractions of the distillate.

In cases where two immiscible liquids are distilled together, the b. p. is the temperature at which the sum of the vapour-tensions is equal to the atmospheric pressure. Thus benzene and water distil together at 69.1°, at which temperature benzene vapour has a tension of 533.7 mm., and steam 224.2 mm., the sum of the two being 577.9 mm.

During the past few years the subject of distillation has been exhaustively studied by Prof. Sydney Young (see especially *Trans.*, **1895**, 679; **1902**, 707, 768; **1903**, 68, 77; Young and Fortey, *Trans.*, **1902**, 717, 739 and 752; **1903**, 45). See his treatise on *Fractional Distillation* (Macmillan & Co., Ltd., 1903).

From a consideration of the foregoing facts it will be evident that a complete separation of a complex liquid into its constituents is never possible by a single fractional distillation, and that in certain cases it is impossible even on repeating the operation a very great number of times.

A great improvement in the practice of fractional distillation was made by Warren, who, in his researches on American petroleum, employed a Liebig's condenser inclined towards the distilling flask, and kept at such a temperature as to cause condensation of the less volatile constituents of the mixed vapour, while those of lower b. p. passed on to a condenser kept cool in the usual way, and inclined in a direction opposite to the first.

Many arrangements have been devised by which the vapour of the distilling liquid is partially condensed and succeeding portions are caused to be washed with the liquid produced, which periodically runs back into the distilling flask. A very useful arrangement of this kind is that of Le Bel and Henninger (Fig. 5) which consists of a number of bulbs, ranging from 2 to 6, blown upon a tube, which is fitted by means of a cork into the mouth of the flask containing the liquid to be distilled. The upper end of the tube is furnished with an inclined side-tube, which can be fitted by a cork to a condenser, and with an

orifice through which a thermometer can be passed, so as to observe the temperature of the vapour which passes over. Each of the bulbs is connected with the one below by means of a small side-tube. In the constriction of each bulb is placed a small cup of platinum or copper gauze, of the size and shape of a small thimble. These cups are made by folding the gauze over the end of a stout glass rod. The ascending vapour condenses in the cups, and thus serves to wash the vapour subsequently formed, as it bubbles through. When the liquid rises to a certain height in each bulb it runs off by the side-tube, and ultimately finds its way back to the distilling flask, the flame
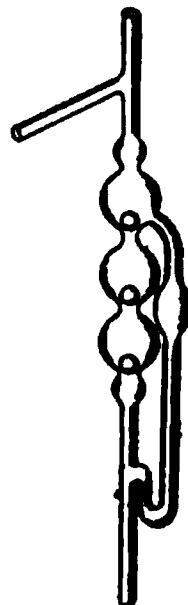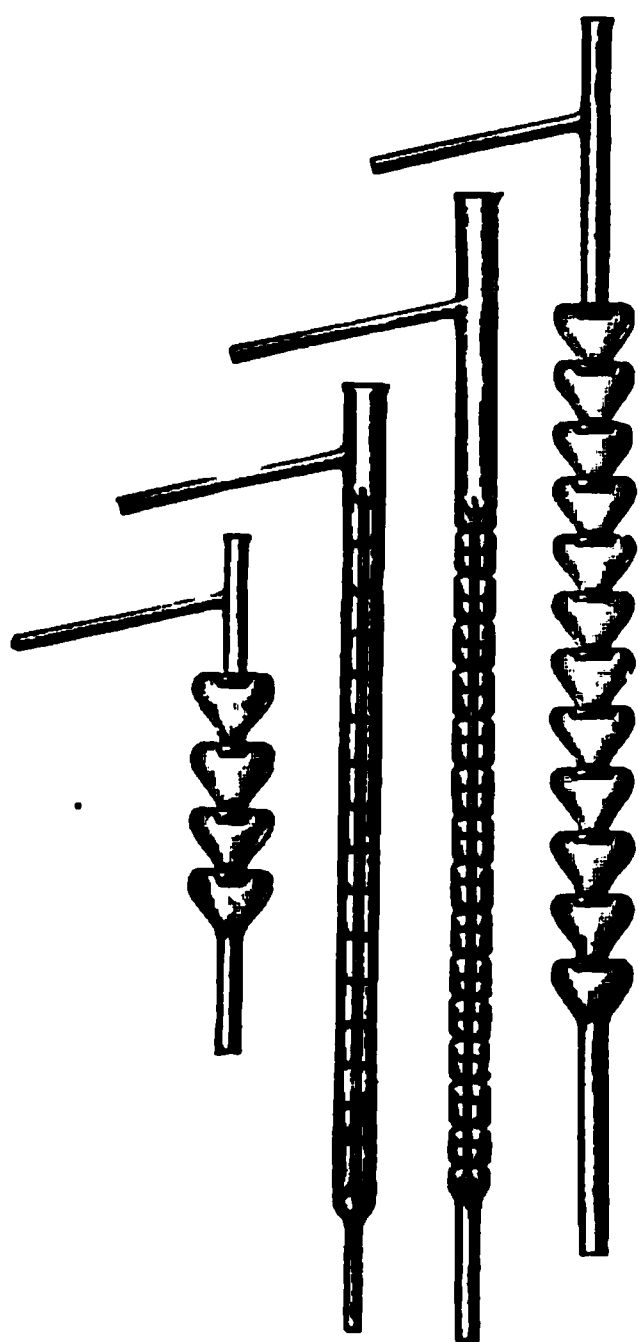

FIG. 5.   FIG. 6.

under which is so regulated as to keep all the cups full, and cause the distillate to fall from the end of the tube in separate drops. In an improved form of dephlegmator, devised by Glynsky (Fig. 6), the wire gauze is replaced by hollow balls of glass, introduced into the bulbs during manufacture.

Hempel (*Zeit. Anal. Chem.*, 1881, 20, 502) substituted for the more complex arrangement a long wide glass tube, arranged vertically and filled with solid glass beads. By this contrivance he obtained alcohol of 95% by slowly distilling spirit of 18%.

For a comparative study of the efficiency of different types of still-head see a paper by S. Young (*Trans.*, 1899, 75, 679), in which new forms are also described. The types of still-head (made by J. J. Griffin & Sons, London) shown in Fig. 7, are very efficient. When a substance decomposes on boiling under ordinary pressure, it can often be purified by distillation under reduced pressure. For methods see Gattermann's *Practical Methods of Organic*

FIG. 7.

Chemistry, and, in greater detail, Lassar-Cohn's *Arbeitsmethoden f. organisch-chemische Laboratorien*, 1903.

OPTICAL PROPERTIES.

Refraction and Dispersion.—The refractive index of a liquid is often a valuable means of identification. The most convenient instrument for accurately measuring refractive indices is Pulfrich's refractometer made by the Zeiss company. For detailed description and instructions for use see Findlay's *Practical Physical Chemistry*. Special types of instrument for measuring the refractive index of butter fat, milk fat, or beer are manufactured by the firm of Zeiss.

REFRACTOMETERS.

As the refractometer is most widely used in analysis in dealing with fats and oils, the description of the different types of this instrument

FIG. 8.

will be included in Volume II. A few of the principal types of refractometer are shown below.

Fig. 8 shows the Pulfrich instrument, made by the Zeiss company, and used for measurements of the refractive index of liquids and solutions. It is fully described in a pamphlet issued by the makers and in most works on elementary physico-chemical measurements, *e. g.*, Findlay's "*Practical Physical Chemistry*" (Longmans). Fig. 9 shows

FIG. 9.

the Abbé refractometer which is used for liquids having a refractive index between = 1.3 and 1.7.

In this instrument the liquid to be tested is placed between two similar prisms which must be of greater refractive index than the sample. When light meets the surface separating the lower prism from the liquid, it is totally reflected if the angle of incidence is greater than the critical angle. Hence, if the double prism be viewed through a telescope the

field will be partly dark and partly bright. The telescope is attached to a sector bearing a scale, while the double prism is connected with an arm which carries an index moving over the divided scale. By rotating the prisms the critical line dividing the field of view can be brought to coincidence with the centre of the cross-webs in the eye-piece. The reading on the scale then gives the index of refraction without any calculation

FIG. 10.

If the light used is not monochromatic the position of the critical line in the field will vary for each component colour. There will thus be a coloured fringe dividing the two portions. In order to annul this colour disturbance a compensator is introduced which consists of two direct-vision prisms of equal dispersion, and can be rotated by means of a screw in opposite directions round an axis parallel to the line of vision. The reading on the divided head of the compensator when the colour fringe is neutralised gives the dispersion of the liquid for the particular light used.

Fig 9 shows the form of instrument used when it is required to maintain the test liquid at a certain temperature. The prisms are mounted in metal boxes, through which a stream of water at the requisite temperature is passed from a convenient source. The Abbé

refractometer with non-heating prisms (Fig. 10) is used in measuring refractive indices of solid bodies (*e. g.*, crystals) and of viscous plastic substances. The measurements are made by means of either reflected or grazing incident light. The Zeiss company supplies on application a pamphlet describing in detail the construction and use of the Abbé refractometers; a special pamphlet giving a complete

FIG. 11.

bibliography of the technical application of refractometers is also issued by the same firm.

As the *Immersion Refractometer* of Zeiss is now very widely used in commercial organic analysis (for example, in the estimation of alcohol in beers by Ackermann's method; in the examination of products containing grape or cane sugar; in the analysis of blood and exudations), a short account may be given of this instrument, which is

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illustrated in Fig. 11. The method of measurement is founded on the observation of the border line of total reflection in a telescope as in the refractometers mentioned a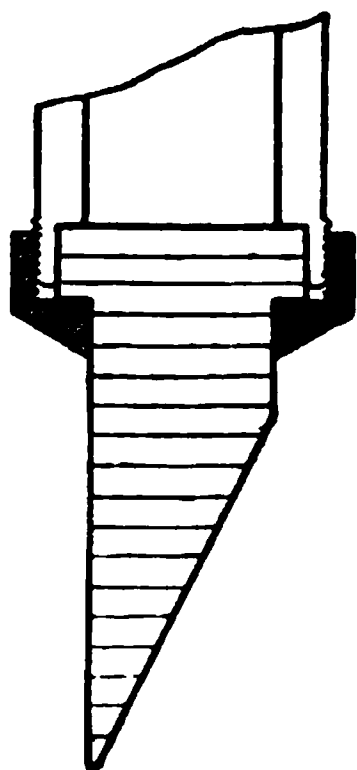bove, but the manipulation is much more simple than with these instruments. The prism (Fig. 12) at the lower end of the refractometer held vertically is simply immersed in the solution contained in a well-filled beaker.

The prism body is cylindrical in shape (so that awkward corners and indentations difficult to keep clean do not exist) and only glass parts are immersed, so that the instrument can be used for acid solutions (for example, acetic acid). In using the instrument care must be taken that day- or lamp-light passes into the fluid parallel to the oblique prism surface, as indicated in Fig. 13, which shows in section an old type of the instrument now superseded by that of Fig. 11. In the new type of dipping instrument, light is reflected from the mirror below the trough.

FIG. 12.

The lower end of the refractometer is immersed in the middlemost of the 5 beakers of the front row. The rectangular mirror fitted under the trough reflects the light of the bright sky through a glass plate upwards into the beaker and through the fluid into the refractometer. The latter hangs by its hook H upon the wire frame T. Observations are made from above by means of the ocular Oc. The border line of total reflection is achromatised by turning the milled ring R. The micrometer screw Z gives one-tenth scale divisions.

Conforming to the new process of observation, the instrument consists essentially of the following parts:

A *Prism* P of hard glass, with a refracting angle of about 63°.

A *Telescope*, rigidly connected with the prism, formed by the objective O and eye-piece Oc, *with the Scale* Sc and *Micrometer Screw* Z in Fig. 12.

A *Compensator* A, placed between prism P and objective O, which can be rotated about the axis of the telescope by means of the milled *Ring R.*

The Border Line, which separates the bright part of the field from the dark, is, on account of the difference in dispersion between glass and fluids, generally fringed with colour and quite unsuitable for an exact reading. On rotating the compensator by means of the milled ring R the colour disappears and the separating line between dark and light becomes quite sharp and colourless. The position of this sharp line

relative to the scale is the measure of the refractive index of the fluid. Table IV gives the value of the refractive index corresponding with each scale division. The scale divisions are read directly and intermediate positions calculated in decimals of a division by means of the micrometer screw Z, the scale being slid across the border line until

FIG. 13.

the scale division previously noted stands contiguous with it. The division on the micrometer drum then shows the decimal of the scale index on the added.

The immersion refractometer has a range of measurement between a refractive index $n_D = 1.325$ (sea-water in the tropics) and $n_D = 1.366$ (alcohol). Within this range fall the refractive indices of aqueous solutions of salts, acids, sugars, and of liquids, such as beer and wine. In

spite of its simplicity, this refractometer excels in the accuracy of its readings all other kinds of refractometer, with the exception of interference refractometers. It is of course necessary that observations should be made at a known temperature, 17.5° being the temperature corresponding with the values in the table.

TABLE IV.

Table for the Calculation of the Scale Divisions of the Immersion Refractometer in Refractive Indices n_D and vice versa.

Scale Divisions	$n_D = 1.3$	Scale Divisions	$n_D = 1.3$
—5	25.39	50	46.50
—4	25.78	51	46.87
—3	26.18	52	47.24
—2	26.57	53	47.61
—1	26.96	54	47.98
0	27.36	55	48.36
1	27.75	56	48.73
2	28.14	57	49.10
3	28.54	58	49.47
4	28.93	59	49.84
5	29.32	60	50.21
6	29.71	61	50.58
7	30.10	62	50.95
8	30.49	63	51.32
9	30.87	64	51.69
10	31.26	65	52.05
11	31.65	66	52.42
12	32.04	67	52.79
13	32.42	68	53.16
14	32.81	69	53.52
15	33.20	70	53.88
16	33.58	71	54.25
17	33.97	72	54.61
18	34.35	73	54.97
19	34.74	74	55.33
20	35.13	75	55.69
21	35.51	76	56.06
22	35.90	77	56.42
23	36.28	78	56.78
24	36.67	79	57.14
25	37.05	80	57.50
26	37.43	81	57.86
27	37.81	82	58.22
28	38.20	83	58.58
29	38.58	84	58.94
30	38.96	85	59.30
31	39.34	86	59.66
32	39.72	87	60.02
33	40.10	88	60.38
34	40.48	89	60.74

Interpolation columns:

40		39		37		36	
1	4,0	1	3,9	1	3,7	1	3,6
2	8,0	2	7,8	2	7,4	2	7,2
3	12,0	3	11,7	3	11,1	3	10,8
4	16,0	4	15,6	4	14,8	4	14,4
5	20,0	5	19,5	5	18,5	5	18,0
6	24,0	6	23,4	6	22,2	6	21,6
7	28,0	7	27,3	7	25,9	7	25,2
8	32,0	8	31,2	8	29,6	8	28,8
9	36,0	9	35,1	9	33,3	9	32,4

TABLE IV.—*Continued.*

Scale Divisions	$n_D - 1.3$		38	Scale Divisions	$n_D - 1.3$		35
35	40.86			90	61.09		
36	41.24			91	61.45		
37	41.62			92	61.81		
38	41.99	1	3,8	93	62.17	1	3,5
39	42.37	2	7,6	94	62.52	2	7,0
40	42.75	3	11,4	95	62.87	3	10,5
41	43.13	4	15,2	96	63.23	4	14,0
42	43.50	5	19,0	97	63.59	5	17,5
43	43.88	6	22,8	98	63.94	6	21,0
44	44.26	7	26,6	99	64.29	7	24,5
45	44.63	8	30,4	100	64.64	8	28,0
46	45.00	9	34,2	101	65.00	9	31,5
47	45.37			102	65.35		
48	45.75			103	65.70		
49	46.12			104	66.05		
50	46.50			105	66.40		

Example: Scale division 3.1 corresponds to the Refractive Index $n_D = 1.32854 + 0.000039 = 1.32858$.

A convenient type of water heater and water-pressure regulator by means of which the trough A is maintained at this temperature, is shown in Fig. 14, and is described in detail in pamphlets issued by the Zeiss company. The arrangement devised by Lowry for supplying water to polarimeter tubes at a definite temperature can also be used (page 54). The description of the Zeiss heater is as follows:

The spiral heater, about 3.5 metres in length, of stout copper, is enclosed in the space between two telescoping metal cylinders. The inner cylinder is provided with a copper bottom, through which the heated air generated by a bunsen burner, petroleum or spirit lamp is evenly distributed and conducted to the copper pipe.

The top of the spiral heater is connected with the tap C of the heating trough A by a short length of tightly stretched tubing, which should incline upwards in the direction of C. In this way the accumulation of air bubbles, which would otherwise obstruct the uniform flow of water, is checked. By means of the tube D the water is drained off by a glass or metal tube and led to a sink. When desired, the heating apparatus can be thrown out of action by turning off the gas, turning the cock at C and drawing off the water.

It is generally best not to have a too sluggish flow of water, and to obtain a certain approximate temperature first by appropriate manipulation of the source of heat; the temperature is finally adjusted to the

exact degree required, by varying the difference in elevation between the cistern A of the water-pressure regulator and the heating trough A.

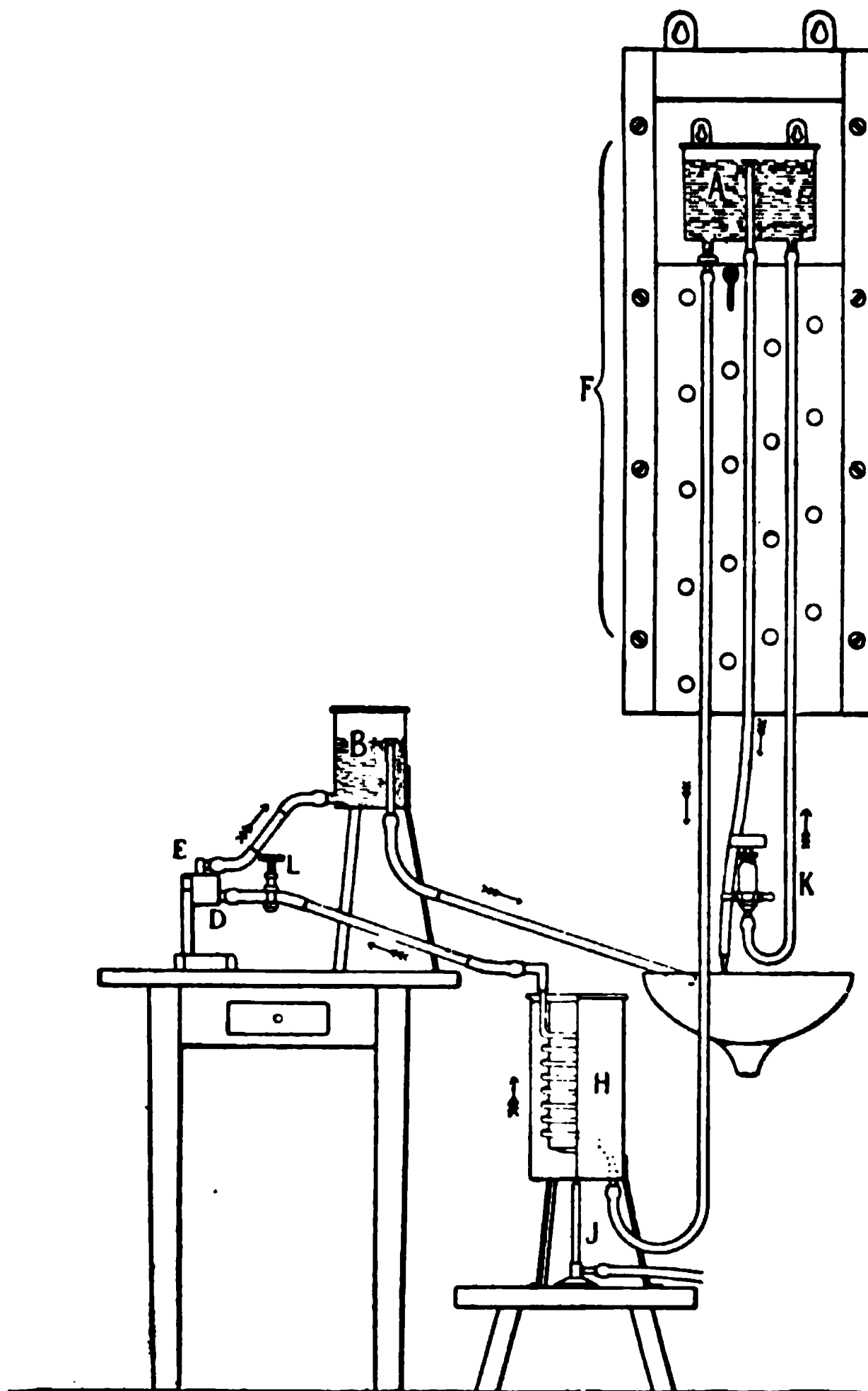The difference in elevation between the cistern A and the heating

FIG. 14.

trough may be varied in two ways: cistern A is either suspended from a cord running on a roller, the free end of which is made fast as with roller blinds, or it is hung on two hooks driven into a board. The

latter can be made to slide up and down in a frame formed of a board about 1 metre in length and fitted with two strips placed lengthwise at the sides, the whole being fastened against a wall. Holes are bored through this board arranged in zig-zag lines and each about 1 cm. above the next lower one, the board with cistern A being kept at the desired height by passing a peg through one of the holes. It is an easy matter to find by trial the number of holes by which the board requires moving in order to cause the temperature to vary by 1°.

Manipulation of the Immersion Refractometer.—It is first necessary to see that the instrument is properly adjusted. For this purpose the heating trough A (Fig. 11) is placed with its long side parallel to the window and the mirror turned towards a bright sky. The trough is then half-filled with water and a beaker filled with distilled water is placed in one of the five holes in the front row immediately above the mirror. Finally the refractometer is hung by the hook H upon the wire frame so that the prism is completely submerged in the water contained in the beaker.

The whole apparatus is now allowed to stand for 10 minutes or so to bring everything to the same temperature. When the distilled water has exactly taken the temperature of the bath, the eyepiece is focussed on the divisions of the scale by turning the milled head of the eye-piece until the lines and numbers are seen quite distinctly, and the mirror adjusted so that the light of the bright sky is seen directed *through the beaker*. The upper part of the field, from —5 to about 15 appears bright and is separated from the lower dark part by a sharp line of demarcation, if the index on the ring of the compensator stands at 5.

TABLE V.

The correctly adjusted refractometer should show, for distilled water at

Temperature . .	10° C.	11	12	13	14	15	16	17	17.5	18	19° C.
the Scale Division	16.3	16.15	16.0	15.85	15.7	15.5	15.3	15.1	15.0	14.9	14.7

Temperature . .	20° C.	21	22	23	24	25	26	27	28	29	30° C.
the Scale Division	14.5	14.25	14.0	13.75	13.5	13.25	13.0	12.7	12.4	12.1	11.8

The reading is taken as already explained and the temperature of the distilled water noted. Reference to Table V[1] will show if the Refractometer be correctly adjusted.

By means of this table it is possible to test the adjustment of the refractometer without having first to adjust the bath to the normal temperature of $17.5°$. Should the average of further careful readings deviate from that contained in Table V, the following should be resorted to:

The eye-piece end of the refractometer, hanging on the wire frame, is grasped from behind with the thumb and fore-finger of the left hand, the micrometer drum set to 10 and the steel point enclosed in the case of the apparatus, inserted into one of the holes of the nickelled cross-holed screw, lying on the inner side of the micrometer drum. The point is then turned *anti-clockwise* (as seen from the rear) whereupon the nickelled milled nut, which governs the micrometer, becomes loosened. The temperature of the distilled water in the beaker is again read to ensure that it has remained constant and then Table V, is consulted to find the "*adjusting number*" properly belonging to the temperature indicated. By turning the point the border line is brought exactly on the integer scale division belonging to the adjusting number, and the still loose micrometer drum is turned so that the index corresponds with the decimal portion of the adjusting number. The drum is now held firmly with the thumb and fore-finger of the left hand, while the *nut* is again screwed up tight by the right hand, care being taken that the drum does not wander off the index. Finally the new adjustment is tested by repeated readings. After the instrument has been properly adjusted, measurements can be made in the manner already described, using a beaker full of the liquid to be examined. When only small quantities of the liquid are available (as, for example, in dealing with blood serum) or the solution is too deeply coloured, as in the case of dark beer or molasses, an *auxiliary prism* is used, the face of which is laid on the polished elliptical face of the refractometer prism. The liquid to be examined is applied between the two prism faces, which are then locked into position by a suitable cover. Details for the use of the auxiliary prism are supplied by the makers of the instrument.

When a volatile liquid or solution which would quickly evaporate

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[1] " Uber quantitative Bestimmungen wässriger Lösungen mit dem Zeiss' schen Eintauch-Refraktometer." By Medizinalassessor, Dr. B. Wagner, Jena, 1903 (Diss.), p. 14.

has to be examined, a metal beaker M in Fig. 13, supplied with the instrument, is used; it is clamped to the prism of the refractometer by means of the bayonet-joint, and, while the refractometer is held with the prism pointing upwards, it is filled with the liquid to be examined. The cap D is then carefully fitted and locked, and the observation made by hanging the refractometer in the wire frame of the trough A, so that the metal beaker is submerged in the bath. If trough B is used (older type of instrument) the refractometer is inclined as shown in Fig. 12.

SPECTROMETERS AND SPECTROGRAPHS.

The Absorption-spectrum of an organic substance occasionally furnishes information not to be obtained in any other way, and in the examination of blood-stains, dye materials, and other coloured substances is often of great utility.

FIG. 15.

Spectrometers and Spectrographs.—Probably the most convenient spectrometer for use in technical analysis, especially in the observation of absorption spectra, is the Hilger wave-length spectrometer of the constant deviation type, made by A. Hilger, London, and shown in Fig. 15.

The prism is of a special form (Fig. 16) and may be considered as built up of two 30° prisms and one right-angled prism from which the

light is internally reflected. Usually the prism is made in one piece, but with very highly refractive glass it is built up of the separate prisms.

The telescope and collimator are always at right angles, being fixed in this position, the different parts of the spectroscopic field being brought across the pointer in the eye-piece of the observation telescope (used instead of cross-wires) by rotating the prism, this being effected by means of a fine steel screw, the point of which presses against a projecting arm on the prism-table. To the screw is fixed a helical
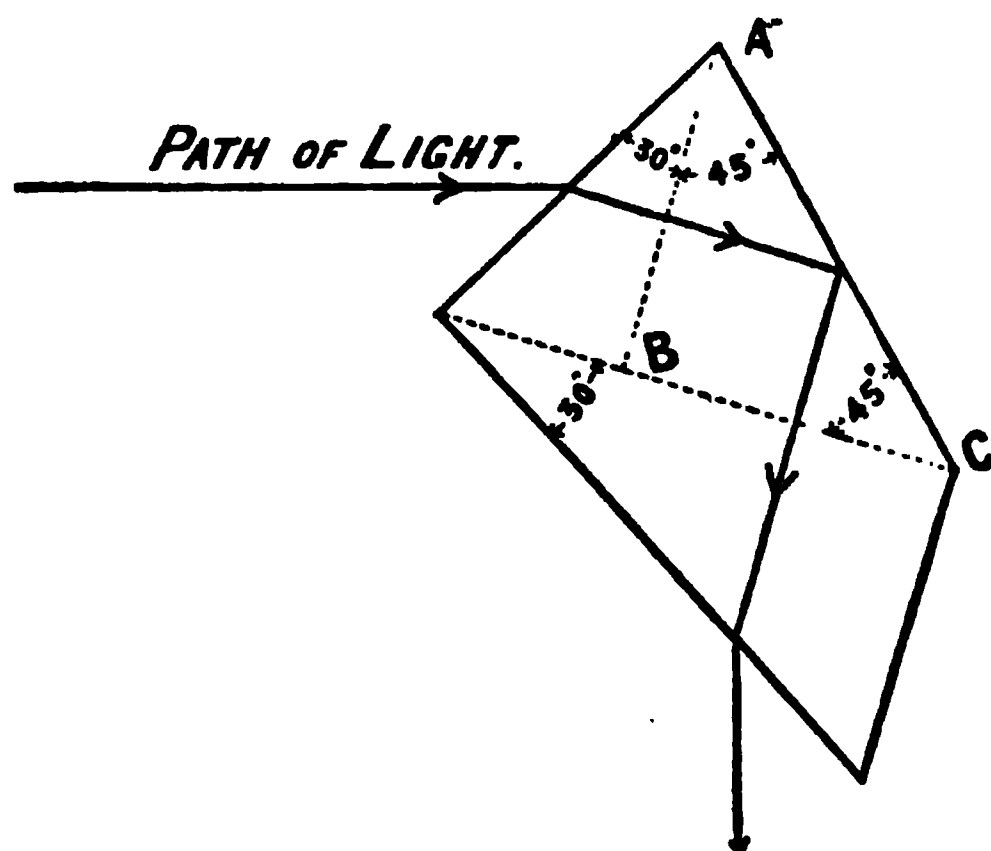

Fig. 16.

drum (see Fig. 17) on which the wave-length of the line under observation and coinciding with the cross-wires in the telescope is read off directly; the wave length being indicated by the index, which runs in a helical slot.

With this instrument, which reads to within 2 Ångström units and in which a single high dispersion prism separates the two D sodium lines by an apparent distance of about 1/32 in., the accurate observation of spectra is enormously simplified. In using the instrument the prism is first fixed in position by reference to one or two lines in the spectrum. For this purpose the drum is first rotated until the pointer corresponds with the wave length of one of the sodium lines (D_1 or D_2); a sodium flame is then put before the collimator, the slit adjusted and the prism rotated carefully by hand, until the sodium line chosen corresponds exactly with the bright pointer in the eye-piece. To simplify the adjustment, the bright pointer itself (shown in Fig. 18) can be moved

laterally by the two milled-head screws below; the metal pointer in the eye-piece is ground exceedingly fine, brightly polished and illuminated from above by the small mirror (Fig. 18). When the position of the prism has been found (an operation which occupies a few minutes only), it is clamped in position by the top screw; its outline should, when the instrument is first used, be marked out by pencil on the base plate. On all subsequent occasions the prism is placed approximately in position by means of this outline and can then be accurately adjusted in

FIG. 17   FIG. 18.

a few moments. When the position of the prism has been fixed by reference to the sodium line the wave-length scale gives the position of every other line in the spectrum. The accuracy of the calibration and of the adjustment of the prism can be tested by reference to any line in the extreme blue; for instance, by means of the blue cæsium line. For this purpose a trace of a cæsium salt is heated on a platinum wire in a bunsen flame.

To observe an absorption spectrum, it is only necessary to place a luminous flame—for example, a Welsbach-burner—before the collimator and to interpose a trough (of a suitable thickness) filled with the liquid under observation. The absorption spectrum is then produced and the wave lengths of the absorption lines or bands can be read off directly on the scale by turning the graduated drum until the absorption bands correspond with the bright pointer in the eye-piece. The two shutters in the eye-piece, which can be moved laterally, are of great service in

observing faint lines, as they can be shifted from either side so as to cover any desired part of the field and thus prevent the eye from becoming fatigued by the glare in the rest of the field.

FIG. 19.

A useful accessory to the above spectrometer is the camera shown in Fig. 19 which is used as a spectrograph.

FIG. 20.

This instrument is of service in recording photographs of absorption spectra.

When absorption occurs in the ultra-violet region of the spectrum it is necessary, in order to obtain photographs of the absorption spec-

trum, that the lenses and prism of the spectrograph be constructed of special glass (ultra-violet glass) which is transparent to the ultra-violet rays; Fig. 20 shows a spectrograph which is suitable for use in obtaining photographs, for example, of the absorption bands of blood.

FIG. 21.

Quartz lenses and prisms have to be used when the extreme ultra-violet region—that is, the region of smallest wave length—has to be examined. Fig. 21 shows a spectrograph with quartz train made by Messrs. Hilger.

FIG. 22.

A much cheaper instrument than any of the foregoing is Hilger's wave-length spectrometer (Fig. 22) of the photographic scale type; measurements can be made with it even more rapidly than with the

drum-reading spectrometer, but it is not quite so accurate. The readings are, however, correct to within about 10 Ångström units.

The photographic scale is mounted on a tube (with collimating lens), and the light from the scale is reflected from the surface of the prism.

A reflected image of the scale is thus seen in the telescope in juxtaposition to the spectrum as shown in Fig. 23 which shows a portion of the complex absorption spectrum of nitric oxide. The dark bands are, of course, not sharply defined in this spectrum. The definition of the scale is very much finer than is shown in the print. The print is the exact size of the real image formed by the telescope object glass. To get an idea of the size of field in the instrument, therefore, the print should be looked at with an eye-piece.

FIG. 23

FIG. 24.

The collimator, prism and photographic scale mount are fixed to a rigid cast-iron base, the telescope alone rotating to pass through the spectrum. To this instrument a camera can also be attached so as to produce photographic records of the spectra under examination.

The Hüfner spectrophotometer (Fig. 24) made by Hilger is designed for the accurate measurement of absorption of liquids for light of any desired wave length. It has found a useful application in the

determination of the densities of photographic plates and, more recently, in the quantitative estimation of minute quantities of nitrogen peroxide, such as are produced by the decomposition of nitro-explosives (see Robertson and Napper, *Trans. Chem. Soc.*, 1907, **91**, 761 and 764). It may here be mentioned that the wave-length spectrometers already described are particularly convenient in carrying out the test for mercury in nitro-explosives, such as cordite. (Compare the article on "Explosives," in Vol. II.)

The Hüfner spectrophotometer consists of the following essentials: It is desired to compare the intensities of two beams of light, one of which has undergone absorption (by passage, for instance, through a known thickness of a liquid under observation). In the path of the beam which has not undergone absorption is interposed a Nicol prism, which polarises the light perpendicularly. The two beams of light are then thrown on the slit of the spectroscopic portion of the apparatus, being brought into close juxtaposition with a sharp dividing line by a prism of special design. The light after passing through the slit undergoes collimation, and is spread into a spectrum by a prism, and after passing through a second Nicol prism is brought to a focus, and observed by an eye-piece. Two spectra are then seen one above the other with a very sharp dividing line between; the one being an absorption spectrum of the liquid substance under observation, the other spectrum being reducible by rotation of the second Nicol prism to any desired intensity. The intensity of this latter spectrum can be simply deduced from the rotation of the second Nicol, and thus, by exact matching of any desired part of the two spectra, an exceedingly accurate measurement of the amount of absorption of the observed material can be obtained. One can pass through the spectrum by a screw motion, with a large drum-head on which the part of the spectrum under observation is marked in wave lengths. Owing to the special form of prism used, the telescope is rigidily fixed as in the constant deviation spectrometer.

The rotation of the second Nicol is read off by a vernier. The eye-piece has two shutters which can be pushed in from right and left, by means of which any part of the spectrum can be isolated. (See page 35, Fig. 18.)

The following works deal with the observation of absorption spectra; Formánek, *Die qualitative Spectralanalyse* (R. Mückenberger, Berlin); E. C. C. Baly, *Spectroscopy* (Longmans). The most exhaus-

tive treatment of the subject is contained in Kayser's comprehensive
"*Handbuch der Spectroscopie.*"

Microspectroscope.—For observing the absorption-spectra of
organic substances a pocket spectroscope will often suffice, but it
is far better to employ a microspectroscope, furnished with a proper
comparison stage and reflecting prism, so as to allow of the spectrum
of the colouring matter under examination being viewed in juxtaposi-
tion with the spectra of standard specimens of known origin.

Fluorescence of organic bodies is a qualitative character often
of much value. It is absolutely necessary that the liquid to be
observed should be *perfectly clear*, as the presence of minute suspended
particles may lead to fallacious conclusions.

As a rule, the phenomenon of fluorescence may be observed by
filling a small test-tube with the fluorescent liquid, holding it in a
vertical position before a window, and observing the liquid from above
against a dark background. Another plan is to make a thick streak of
the liquid on a piece of polished jet or black marble, or on a glass plate
smoked at the back, and to place the streaked surface in front of, and
at right angles to, a well-lighted window. Either of these methods is
superior to the polished tin plate sometimes recommended. The
background should be black, not white.

In some cases, the following method of observing fluorescence may
be advantageously employed. A cell is made by cementing a piece of
barometer-tube about 3/4 in. in length, and having an internal diam-
eter of 1/6 in., to a glass microscope-slide, by means of black sealing-
wax. The open end of the cell must be well polished. On introduc-
ing a clear solution of any fluorescent substance, covering the cell with
a piece of thin glass, placing the slide on the stage of a microscope,
illuminating the tube at the side by means of strong daylight, and
looking down and observing the axis of the cell by a low microscopic
power, the liquid will appear more or less turbid and of a colour
dependent on the nature of the fluorescent substance in solution. If
no fluorescent substance be present, the field will appear perfectly
black, as no light is reflected either from the apparatus or the liquid.
For a sensitive method of detecting fluorescence see Francesconi
and Bargellini, *Atti dei Lincei* 1906, [5] **15,** No. 3. When desired,
the *spectrum* of the fluorescent light can be observed by the micro-
spectroscope. In some instances the spectrum thus obtained shows
remarkable and characteristic bands.

Double refraction, as observed under the microscope by means of polarised light, is often of value for the recognition of organic bodies. In addition to the well-known phenomena dependent on crystalline form, many organic substances not actually crystalline exhibit a cross and series of rings when viewed by polarised light. This is notably the case with many of the starches, and furnishes a valuable means for their discrimination. The optical properties of crystals often serve as a means of identification. For the use of the polarising miscroscope in this connection see Weinschenk, *Anleitung zum Gebrauch des Polarisations-Mikroskops,* Freiburg, 1906.

POLARIMETERS.

Rotation of the Polarised Ray.—Organic substances containing a so-called asymmetric carbon atom possess the power of rotating the plane of polarisation of a luminous ray; as this property is exerted even by the *solutions* of optically active substances, the angle through which the rotation occurs often serves for the accurate estimation of certain compounds   The method is much employed in the examination of saccharine substances.

Construction of Polarimeters.—In all forms of apparatus for measuring the rotation of the plane of polarisation of a luminous ray, the polariser, or optical means of obtaining a beam of polarised light, consists of a double-refracting prism of calcite.   In some cases a double-image prism is used, but in others the extraordinary ray only is employed. The analyser is composed of a Nicol prism, with a suitable eye-piece. On rotating the analyser through 90° the field becomes perfectly dark, but on introducing between the analyser and polariser a tube filled with sugar solution or other optically active liquid the light again passes. With white light the transmitted tint differs with the strength of the solution of sugar and the length of the tube, and rotation of the analyser merely causes an alteration in the colour of the transmitted light, a phenomenon due to the fact that rays of differing refrangibility are rotated unequally (rotatory dispersion). If monochromatic light be employed, a certain angular rotation of the analyser will suffice wholly to extinguish the light from the field of view, and hence, by measuring the angle through which the analyser must be rotated to restore darkness, an estimate of the strength of the interposed liquid in sugar or other active constituent may

be obtained. Quartz possesses powerful rotatory action, a plate 3.75 mm. in thickness (=0.148 in.) rotating the plane of polar isation of the mean yellow ray through 90 degrees. Some specimens of quartz are dextrorotatory, others are lævorotatory. Hence, a double plate composed of equal thicknesses of the two varieties possesses no rotatory power. If a plate composed of semicircles of right- and left-handed quartz, each 3.75 mm. in thickness, is placed between Nicol's prisms, while the principal sections of the latter are parallel, the field assumes a peculiar purple, known as the *transition tint*. The least rotation of the analyser causes one half of the circle to incline to red and the other half to violet, and the interposition of a solution of an optically active substance produces a similar effect, while to restore uniformity of tint necessitates a rotation of the analyser through an angle dependent on the strength and thickness of the polarising liquid used.

Laurent's polarimeter is one of the simplest. One-half of the field of vision is covered by a very thin plate of quartz which causes an alteration in phase of half a wave length and allows light to pass, even when the analyser and polariser (both of which are Nicol's prisms) are crossed. If the analyser be rotated so as to cause the quartz plate to become dark, light passes through the uncovered half of the field. In a position intermediate between these two the two halves of the field appear equally dark, and this is the zero point of the instrument. The slightest deviation from this neutral position causes one half of the field to appear darker and the other half lighter than before. Hence the change is a double one and the instrument is thus made very sensitive. Monochromatic light must be used.

The Lippich polarimeter, now widely used, is also a half-shadow instrument differing from the Laurent polarimeter in the method adopted for producing the half shadow, a small Nicol prism replacing the quartz plate. Triple-field instruments have also been devised. In these the field is divided vertically into three zones, the central one being a broad band. The optical construction is such that the lateral zones always agree in tint, thus making the contrast with the central portion more marked. In one form of instrument the portions of the field are concentric. It is claimed that this method gives a high degree of delicacy (see below).

The use of monochromatic light, *desirable* in saccharimetry, is *essential* in estimating many substances. This is due to the fact that Biot's law, that the angles of rotation for the different simple colours

are proportional to the squares of the indices of refraction and inversely as the squares of the wave-lengths, is true of quartz and saccharine liquids, but does not hold good generally. In all cases, to insure accuracy, not only should monochromatic light be employed but the liquid under observation be kept at a known temperature.

For monochromatic light, the lamp usually employed is a bunsen burner with a ledge at the top for holding some solid sodium compound. A fused mixture of sodium chloride and phosphate is better than sodium chloride alone. The following is an excellent method for obtaining a steady, strong yellow light: Strips of common filter-paper 5 cm. wide and about 50 cm. long are soaked in a strong solution of sodium chloride and thiosulphate, dried, and rolled into a hollow cylinder of such size as to fit firmly on the top of the burner. The cylinder is kept from unrolling by a few turns of fine iron wire. The flame burns at the top of the cylinder, giving for the first few minutes a luminous cone, but soon becoming pure yellow. The cylinder becomes a friable charred mass, but if not disturbed may be used for some time continuously or at intervals.

An effective method for producing a sodium flame is that devised by Caldwell and Whymper (*Proc. Roy. Soc.*, 1908, **A 81**, 112-117). A Mecker burner is taken off the ordinary base, screwed to a piece of brass tubing and fixed in an ordinary glass bottle by means of a rubber stopper. The gas supply is led into the bottle by a glass tube and passes through a powder consisting of an intimate mixture of finely ground dry sodium carbonate and clean sand. The sand is necessary to prevent the particles of sodium carbonate from caking. So much sodium carbonate is blown up that the whole flame (6 by 1¼ inches) is uniformly coloured an intense yellow. It has about 60 candle power.

H. W. Wiley has pointed out the usefulness of acetylene as a source of light for polarimetric work. By the use of this light he was able to make readings through liquids which were too dark to permit light from ordinary sources to pass. Since acetylene can be readily and safely prepared by self-regulating apparatus, it will doubtless find application in this and in other departments of laboratory work. Landolt recommends an Aron's mercury vapour lamp as a convenient means of obtaining monochromatic light in polarimetry. Lowry has employed (*Proc. Roy. Soc.*, Nov. 19, 1908), the Bastian mercury lamp with great advantage in measuring rotatory dispersion.

Before using the polarimeter the observing tube should be filled with distilled water and placed in position between the polarising and analysing prisms, which are then to be adjusted, so that the latter shall be at the zero point of the scale when there is no optical disturbance of the field. The tube is then filled with the solution to be tested and replaced between the polariser and analyser, when, if it contain an active substance, an optical disturbance will be observed, the extent and direction of which will depend on the amount and nature of the rotating substance under examination. The polarimeter is then adjusted, so that the neutral point is reached, or, in other words, so that the optical disturbance produced by the introduction of the rotating liquid is compensated; the rotation required to produce this effect is then read and recorded. From the circular rotation observed, the specific rotatory power of the substance may be calculated in the manner described in the next paragraph.

Full directions for the preparation of the solution and the practical management of the polarimeter will be found in the section on the "Sugars."

Specific Rotatory Power.—The specific rotatory power of an optically active substance is the angular rotation exerted by it on a ray of polarised light traversing a thickness of 1 decimetre (=3.937 ins.) of the substance.

The *absolute* specific rotatory power of a solid can only be observed by using thick slices of considerable transparency. It is usual to operate on a solution of known concentration, and from the *sensible* or *apparent* specific rotatory power observed, to calculate the *absolute* rotatory power of the solid substance.

The apparent specific rotatory power of a substance in solution [a] is obtained from the following measurements:

a = The observed angle of rotation in degrees.
c = The concentration of the solution is grm. per 100 c.c.
L = The length of the column of solution in mm.

$$[a] = \frac{10^4.a.}{Lc.}$$

The apparent specific rotatory power of a substance varies greatly with the wave length of the light employed (rotatory dispersion); it is therefore necessary to make the measurements with monochromatic light of one particular wave length and to state the position in the

spectrum of the particular ray employed. In practice the rotation of a substance is expressed in two ways. Either it is referred to the D line of the solar spectrum, the rotation being then expressed by $[a]_D$; or it is referred to the "medium yellow ray" (*jaune moyen*) which is complementary to Biot's transition tint; the rotation being then denoted by $[a]_j$. In the former case $[a]_D$ is measured by a Wild, Mitscherlich, Jellet-Cornu or Laurent instrument and the direct rotation is found in degrees of arc. In the latter case, $[a]_j$ is measured by means of a neutral-tint or half-shadow polarimeter, such as the Ventzke-Scheibler. The scale divisions in such instruments are arbitrary and have to be converted into angular degrees before the specific rotatory power can be calculated. The readings obtained being based on the rotation of a quartz plate are obtained in terms of the rotation of a quartz plate of definite thickness. Whilst the rotatory dispersion of quartz and canesugar solutions are nearly identical, most other substances have a very different rotatory dispersion from that of quartz. Thus, a quartz compensating instrument can only be used in the comparison of rotatory powers of different substances, when the rotatory dispersion of the substance under examination is known relatively to that of quartz.

The wave length of the "mean yellow" ray being less than that of the D line, the numerical value of $[a]_j$ is greater than that of $[a]_D$. With a quartz plate 1 mm. thick, Broch found

$$[a]_D = 21.67$$
$$[a]_j = 24.5$$

Whence: $[a]_j = 1.1306 \ [a]_D$ or approximately 9/8 $[a]_D$.

$[a]_D = 0.8845 \ [a]_j$ or approximately 8/9 $[a]_j$.

The proportion between $[a]_j$ and $[a]_D$ varies in different substances owing to their having a different rotatory dispersion.

For sugar solutions $[a]_j : [a]_D = 1.129$.
  (Very nearly the same as for quartz.)

For camphor solutions in alcohol the ratio is 1.198 and for oil of turpentine 1.243.

Use of the Polarimeter.—Fig. 25 shows a Schmidt & Haensch polarimeter of the Lippich half-shadow type with divided circle reading by magnification to 0.01°. It is intended for use with a sodium flame, a gas burner for this purpose being supplied with the instrument. This instrument is chosen as being a typical half-shadow

instrument and is now in general use.   For a complete account of the
construction of the many types of polarimeters, the influence exercised
by solvents ánd temperature in the specific rotatory power, Landolt's
treatise, *"Das optische Drehungsvermögen"* (F. Vieweg und Sohn,
Braunschweig, should be consulted.   An English translation has been
made by J. H. Long.[1]

Fio. 25.

In the above illustration F indicates the telescope, *l l* the magnifying
glasses, *n n* the two verniers, K the graduated dial, A the analysing
Nicol prism, which is fixed to the revolving graduated dial and to the
telescope, and P the movable polariser, with the graduated segment *h* of
a circle fixed to it, and S a small tube for dichromate solution.
The sodium lamp is placed at a distance of 36 cm. from the appa-
ratus.   It consists of a Bunsen burner (or a Barthel's spirit burner)
supplied with a platinum ring on which some pulverised sodium chlo-
ride is placed and made intensely incandescent by means of the non-

[1] For a useful, concise description of many types of polarimeter and details of manipula-
tion, Messrs. Baird & Tatlock's catalogue may be consulted with advantage.

luminous flame from the burner; the apparatus is pointed towards the brightest part of the yellow flame, which can easily be accomplished by means of the adjuster provided with the lamp.

The graduated dial, which is made to revolve by means of a knob T, is as a rule graduated all the way round. In addition to whole degrees, half and quarter degrees are indicated on the dial; 24 such quarter degrees are divided on the 2 verniers into 25 divisions, therefore a scale mark on the vernier coinciding with any one scale mark on the dial indicates 0.01°. Fig. 26 shows the inner revolving dial and the exterior vernier; the zero line of the vernier is shown between the 13.50 and the 13.75° line of the dial; the 0.16 of the vernier, coincides with a line on the dial, therefore the total reading is 13.50+0.16=13.66°.

FIG. 26.

If desired, a second scale can be provided on the dial to show directly percentage of some other sugar. This is done by dividing the dial into whole percentages; nine such are divided on the vernier into 10 divisions, so that the vernier reads to 0.1 per cent. The reading is made in the same manner as described above; the beet-sugar scale is based on the standard weight of 26.048 grm. The 100 line 100%) corresponds with a solution of 26.048 grm. of chemically pure sugar in a 100 c.c. flask, examined with the 200 mm. tube. The so-called grape-sugar scale is now rarely used, but if a tube of a certain length (according to the most recent researches 189.4 mm.) is used in connection with the degree scale, the percentages can also be found directly, one degree in this case corresponding exactly to 1% of so-called "grape sugar."

The adjustment.—When the above apparatus is well illuminated by the sodium flame, the zero position (the starting-point of all experiments) must first be found; this is indicated by the two halves of the field appearing equally illuminated (equal half-shadows). For this purpose the telescope F is focussed on the Lippich's polariser, so that the field presents a perfectly clear, round circle divided into two equal parts by a sharply defined vertical line. If the graduated dial is turned through 3 or 4 degrees to either the right or the left of the zero line,
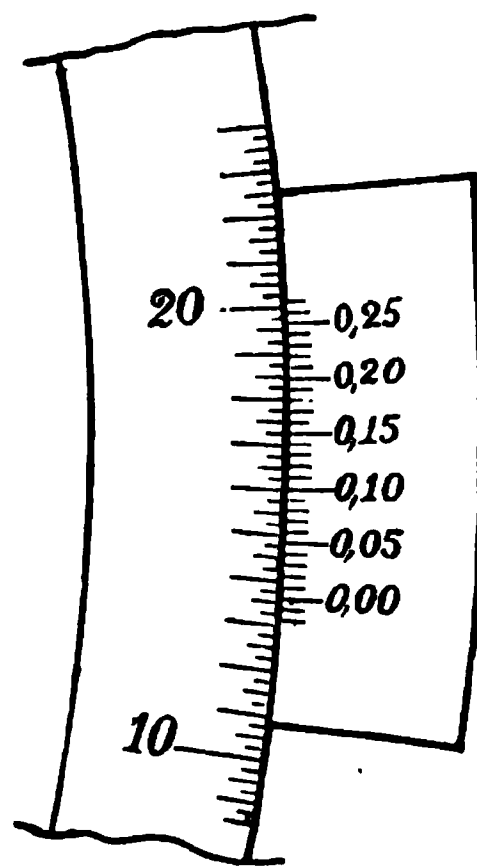
it will be seen that one-half of the field will become lighter, the other half darker.

The zero position is first adjusted so that the zero line of the circle coincides with the zero line of the vernier. The half-shadow can now be made lighter or darker, according as the polariser is turned to right or left of the zero line by means of the pointer h. When the pointer h is at zero and the analyser A also at zero, both halves of the field appear black. The nearer the pointer is to the zero line the darker the half-shadow and the more sensitive the apparatus. In cases when the solution is not quite transparent, the pointer must be moved slightly away from the zero line so that the field is clear. The instrument is usually so adjusted that the position of the pointer is at $7\frac{1}{2}°$ when the disc and vernier read exactly $0°$. When the pointer is moved the zero of the apparatus changes and no longer corresponds with the zero of the dial. The simplest method is then to take into account the difference in the position of the zero line of the dial and the zero line of the apparatus; or the graduated dial is moved to $0°$ and the apparatus placed in the zero position by turning the analysing Nicol by the screw A to the right or left until the half-shadows are equal in tint.

The following points must be especially observed during a measurement:

1. When the circle has been turned too far and the sensitive range of the apparatus has been lost it is easy to mistake the zero position owing to the light appearing nearly of the same intensity on both sides of the vertical line. Even if the circle is then turned through 10 or $15°$ hardly any change will be observed. It is, therefore important, especially when the sample under examination has been placed within the apparatus, to make sure that the transition from light to shade, and *vice versa*, is instantaneous when the circle is turned a few degrees on either side of the zero.

2. When the sample to be tested is inserted, the telescope must first of all be adjusted accurately so that the field is quire clear and equally divided by the vertical line; the circle is then turned until the shades are exactly of the same intensity in the two portions of the field.

Precautions to be Observed.—Before the polarimeter tube is filled it should be thoroughly dried by pushing a plug of filter-paper or cotton wool through it, or it should be rinsed several times with the solution to be used. The cover-glasses must be free from scratches and thoroughly clean and dry. Unnecessary warming by the heat of the hand

during filling should be avoided; the tube is closed at one end by the screw-cap and cover-glass and grasped at the other with the thumb and finger. The tube is filled with the solution until the meniscus projects slightly above the opening, the air bubbles are allowed time to rise and the cover-glass then pushed horizontally over the end of the tube in such a way that the excess of liquid is carried over the side, leaving the cover-glass exactly closing the tube without air bubbles beneath it and without any liquid on its upper surface. After the cover-glass is in position the tube is closed by screwing on the cap, care being taken that too great a pressure is not exerted, for this might pro-

duce a rotatory power in the glass itself and thus give rise to erroneous readings. The rubber washers must therefore be placed in a proper position and the caps screwed in lightly.

Before taking the actual reading, observations are made of the zero and with a standard quartz plate of known rotation. The mean of several readings is taken and corrected for any deviation of the zero.

In the polarisation of the quartz plates and colourless solutions, difficulty may be experienced in obtaining a complete correspondence
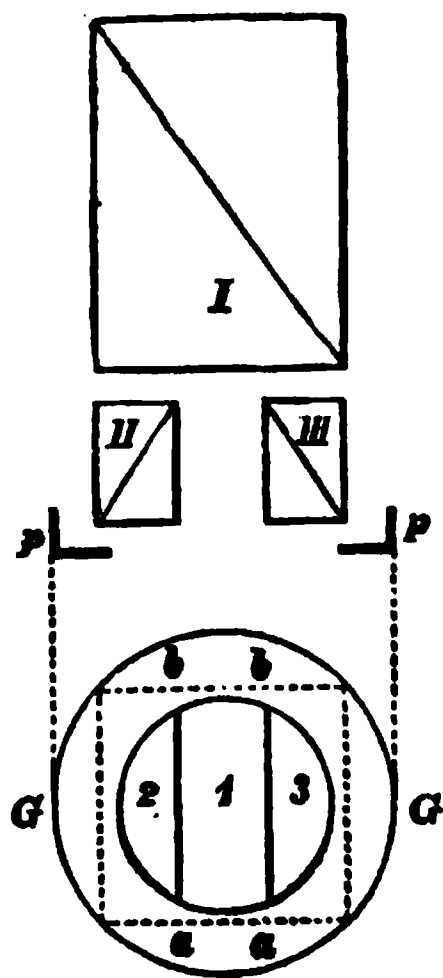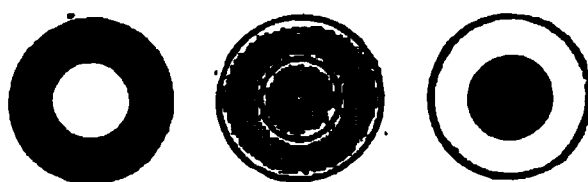

FIG. 27.

FIG. 28.

of both halves of the field. This may be overcome and the neutral point found, but when it cannot, the ordinary eye-piece of the instrument may be replaced by another which is supplied with the polariscope, and which carries a section of a crystal of potassium dichromate. This removes the difficulty and renders it possible to obtain a field of exact neutrality.

In the latest types of polarimeter the optical field is divided into three parts instead of two as in the half-shadow instruments. Such instruments are more accurate, the equality of the field being capable of a more delicate adjustment.

. The arrangement is that shown in Fig. 27. In the zero position 1, 2 and 3 are equally illuminated while in any other position 1 is dark, while 2 and 3 are illuminated or 1 is bright and 2 and 3 equally dark.

Ring-shadow polarimeters (Fig. 28) are a modification of the half-shadow instruments.

They are used in precisely the same way as the ordinary half-shadow polarimeters, but the field of view is divided into two concentric portions instead of into two semicircular segments. With this arrangement the instrument is capable of finer adjustment and the eye is much less fatigued than when using the half-shadow polariser.

Fig. 29 shows an improved form (German patent) of tube used for holding the solution to be examined in the polarimeter. Air bubbles
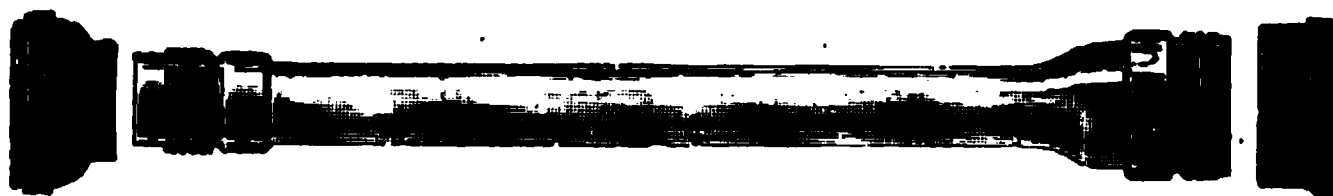

FIG. 29.

enclosed in the tube disappear into the enlargement *a* at one end of the tube and so cease to give trouble during the observation. The caps are of brass and are screwed on as shown. This pattern of tube is particularly useful in dealing with volatile liquids, such as chloroform.

A specially cheap form of half-shadow polarimeter of the Mitscherlich type in shown in Fig. 30, which reads to 0.1° and has been designed for the estimation of sugar and albumin in urine.

Behind the analyser is a small telescope, and behind the polariser a semicircular plate of quartz. The telescope is focussed on to this plate, and the field of vision appears as a circle divided into two halves. A pointer is attached to the analyser, which moves to the right or left on a metal disc divided into angular degrees. A vernier upon which 10 divisions correspond to 9 divisions of the disc enables the observer to read tenths of an angular degree and estimate twentieths.

The instrument is constructed for monochromatic light. A sodium lamp must therefore be used as the source of illumination. The zero point, as in other half-shadow instruments, is found when both halves of the field are of the same tint.

The tube filled with the liquid to be examined is placed in the instrument, and after having focussed the plate by means of the telescope, the pointer is turned to the right or left according to whether the solu-

tion is dextro- or lævorotatory, until both halves of the disc are again equally tinted.

If the instrument is to be used for general work, a tube of the length of 200 mm. is supplied, and another of 100 mm. for dark coloured solutions, but when used exclusively for urine it is more convenient to have one of 189.4 mm. and another of half that length. These tubes give at once the percentage by volume of sugar and albumin,
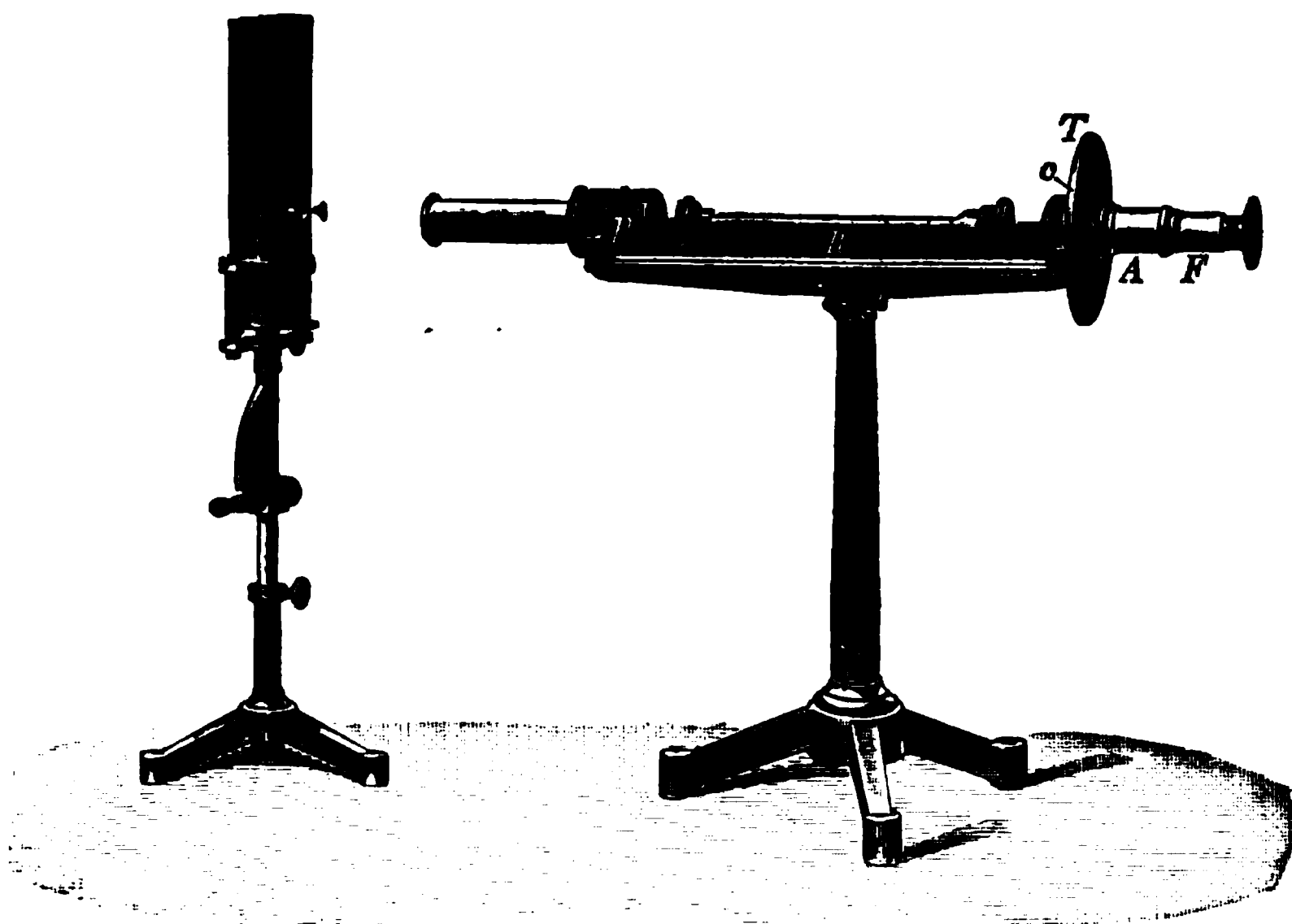
FIG. 30.

each degree being equal to 1 grm. in 100 cc. Albumin polarises to the left to the same extent as glucose does to the right.

The estimation of sugar and albumin in urine is effected in the following manner: The urine, if necessary, is filtered. Should it be too dark coloured to be read in the long tube, the short one is tried, and if still too dark, some extracted animal charcoal is added, and the whole well shaken. In the event of this not effecting decolourisation, 100 c.c. of the urine is introduced into a flask graduated to contain 100 and 110 c.c., basic acetate of lead is added to the 110 c.c. mark, the mixture is then shaken and filtered, and the reading multiplied by 0.11 to correct for dilution. The temperature should be 15° to 20° C. If the urine is free from albumin the reading corresponds to the percentage of

sugar.   Should it contain albumin, a few drops of acetic acid are added
to 100 c.c. and the solution boiled, cooled, filtered, and made up again
to volume at 15° to 20° C.

As it is necessary to maintain a known constant temperature in order
that accurate and comparable measurements can be made with the
different types of polarimeters, water or steam jacketed tubes are sup-
plied by the different makers in which the solution to be examined can be

FIG. 31.

heated at an approximately constant temperature.   Full information
as to these are generally given in the catalogues of dealers in scientific
apparatus.   The special form of thermostat due to Lowry, which is
described in detail elsewhere (page 55), enables a flow of water to be
constantly circulated through a polarimeter tube at a temperature
which can be maintained constant to within a few thousandths of a
degree.

Fig. 31 shows a polarimeter made by Hilger, of London, taking
tubes 200 mm. in length.   The field of view is of the following form
(Fig. 32), in which the illumination of the middle strip decreases in in-

tensity when the outer increases, and *vice versa*. The brightness of the illumination can be varied by rotation of the polariser; an index and clamp are provided for the setting of this adjustment.

The table showing the apparent specific rotatory powers of different organic substances which was included in previous editions of this work has been omitted in the present edition, because the value of the specific rotatory power of a substance differs widely with the solvent used and with the temperature and concentration of the solution; numbers expressing the rotatory power are, therefore, misleading unless the exact conditions observed in measurement are specified. For details concerning all questions of polarimetry and a discussion of the influence exerted by solvents, etc., Landolt's "*Optische Drehungsvermögen* should be consulted. All available numerical data for the rotatory power of organic substances are included in that work.

Fig. 32.

Comparison of Scales of Various Instruments.—Polarimeters are now usually provided with a scale reading to 100 when a certain quantity of sucrose, called the normal weight, is dissolved in water and made up to 100 c.c. This scale is known as "Ventzke," "Schmidt and Hænsch," and "sugar" scale.

The following factors may be employed for the conversion of data obtained by different instruments:

1 division Schmidt and Hænsch (Ventzke) 0.3468° angular rotation D.
1° angular rotation D   2.8835 divisions Schmidt and Hænsch.
1° angular rotation D   0.7551 division Wild.
1 division Laurent   0.2167° angular rotation D.
1° angular rotation D   4.6154 divisions Laurent.

ARRANGEMENTS FOR MAINTAINING A KNOWN CONSTANT TEMPERATURE.

Several types of thermostat have been devised for the purpose of making physico-chemical measurements in a bath at a known temperature; these are described in treatises on physical chemistry (*e. g.*, Ostwald-Luther, *Physiko-chemische Messungen;* Findlay, *Practical Physical Chemistry*). Lowry (*Trans.*, 1905, **87**, 1030 to 1034) gives an

V

FIG. 33.

account of a series of tests of gas regulators of different patterns used to control the temperature of a bath containing 30 litres of water and well stirred by a paddle driven by a water motor. Two forms of regulator are described, by means of which a known temperature, $e.$ $g.$, 20°, can be maintained in the bath within a few thousandths of a degree over a long period. In a later paper (Lowry, *Trans. Faraday Society*, 1907, 3) a thermostat is described, by means of which a flow of water can be obtained, suitable for heating a polarimeter tube or refractometer prism at a constant known temperature; even when the rate of flow of the water circulation is 4 litres a minute, the temperature in the bath does not vary by more than a few thousandths of a degree. This form of thermostat is particularly serviceable in the examination of *Explosives*. (See Vol. II.)

This apparatus (made by Messrs. Baird & Tatlock), which the writer has seen in constant use during long periods and which needs practically no attention, is constructed as follows·

The container, as shown in Fig. 33, consists of a large zinc-lined box, 20 × 18 × 16 ins., with a capacity of over 70 litres. The liquid is stirred by a propeller driven by an electric motor. The bulk of well-stirred water and the heat

FIG. 34.

insulation of the wooden box and cover render the regulation of the bath temperature exceptionally easy, with the result that when the gas flame is controlled by a 4-in. spiral (shown at 3 in the figure, and in detail in Fig. 34), the variations are so small that they escape detection even with a thermometer graduated in hundredths of a degree. The heating is effected by means of a small bat's-wing burner placed beneath a copper plate which forms. the bottom of the central well of the water-bath; the supply of gas to the burner is controlled on the one hand by the by-pass tap B, on the other hand by the spiral S.

The bath is provided with an adjustable overflow F; in cold weather it is only necessary to allow an occasional drop of water to drip into
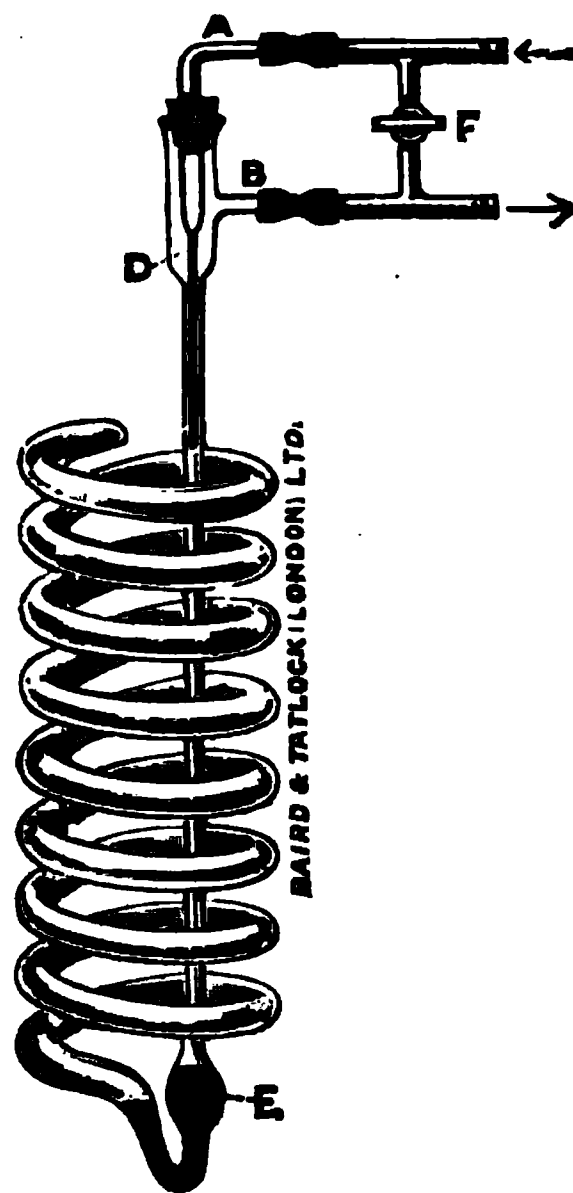
the side tube of the bath to maintain the level, but in summer the flow
is diverted into the bath itself and greatly increased; ideal conditions are
reached when the air temperature is up to 20° and the water tem-
perature a few degrees below. ·

The water circulation is maintained by means of a rotary pump
P ("*Albany*" pump) which *sucks* the water out of the bath from
a point near to the centre of the regulator, draws it through the
various jackets and returns it to the bath. In the figure there are
shown (1) an ordinary Schmidt & Haensch jacketed polarimeter tube
J; (2) a copper water jacket K with a removable lid L, both supplied
with circulating water, designed to take either of the stock patterns of
unjacketed polarimeter tubes; when not under observation these can be
stored in the bath itself in the vertical tubes V or, better, in the hori-
zontal tubes H; the pump may then be stopped, but a slight tempera-
ture gradient (0.1° to 0.2°) will appear in the bath if the stirring is
also discontinued. The temperature of the return flow can be read by
means of a standard thermometer T, graduated in hundredths, which
dips into a tube of mercury round which the circulating water rapidly
passes on its way back to the pump. The rubber bulb R serves to take
up a part of the thrust of the pump; it usually becomes flattened, but
continues to pulsate, when the flow exceeds 1 litre per minute.

The pump is driven from an electric motor M through the gearing G
which carries the propeller and is provided with several adjustments.
The speed of the motor is controlled by a lamp-resistance not shown
in the figure. When this resistance is short-circuited the pump gives
a maximum flow of 4 litres per minute. Under normal conditions a
180-volt, 16-candle-lamp resistance on a 200-volt circuit gives a flow
of about 1 litre per minute. The temperature gradient when the
bath is at 20° and the room at 15° is about 0.01° per jacket.

When the apparatus described above is used for heating polarime-
ter tubes or refractometer prisms, the temperature gradient in the
leads and jackets is reduced to a minimum by the rapid flow of
water which can be increased to any desired extent by speeding up
the pump or increasing its size.

A simple form of apparatus made by the Zeiss comyany for keeping
refractometer apparatus at a constant temperature is described under
Refractometers (page 29).

A simple thermostat by means of which a sp. gr. or measuring flask
can be maintained at a definite temperature, *e.g.*, 15°, 17.5° or 20°, is

constructed by taking a large enamelled iron cylindrical saucepan of a diam. of about 15 ins.; the water contained in this is kept at a constant temperature by means of a 4 in. diameter spiral toluene thermo-regulator, such as is shown in Fig 34. The water in the thermostat is kept stirred by a small paddle, run by a small water or electric motor.

ULTIMATE ANALYSIS.

When organic substances are heated to redness in the air or in the presence of oxygen-yielding substances, they are generally completely oxidised, the carbon being burnt to carbon dioxide and the hydrogen to water. Nitrogen is evolved for the most part in the free state, but in some cases partly in combination with oxygen.

The following general outlines may be of service in enabling a suitable method of analysis to be chosen.

Carbon and hydrogen are estimated by igniting the substance with dry copper oxide with or without the assistance of a stream of oxygen. The resultant water is absorbed by calcium chloride and the carbon dioxide by potassium hydroxide or soda-lime. In presence of sulphur, chlorine, bromine, iodine or light metals, lead chromate is substituted for the copper oxide. Mercury is liable to distil over into the water-absorption apparatus. In presence of nitrogen the anterior part of the tube is filled with metallic copper. Silver may be substituted for the copper, and has the advantage that it retains halogens, but a high temperature should be employed. The Wetzel potash bulbs (Ber., 1903, **36**, 161) are strongly recommended by the writer for the absorption of carbon dioxide (see Fig. 35), owing to their remarkable efficiency. The Hill type of calcium chloride tube (*Proc. Chem. Soc.*, 1906, **22**, 87) is also very convenient.

FIG. 35.

Dennstedt (*Anleitung zur Vereinfachung elementär Analyse für wissenschaftliche und technische Zwecke*, 1903; see the series of papers in the *Berichte*, 1897, **30**, 1590 and 2861; *Zeit. anal. Chem.*, 1902, **41**, 525; 1903, **42**, 417; *Zeit. angew. Chem.*, 1905, **18**, 1134) has de-

vised a method of combustion in which the substance is volatilised in oxygen and the combustion effected by the aid of platinised quartz or, in the later types of apparatus, of special platinum combusters. The great advantage of the apparatus is that comparatively little heating is required to burn the substance completely and the use of a furnace with a large number of burners is avoided. The method has been still further simplified by J. Walker and T. Blackadder (*Proc. Royal Soc. Edinb.*, 1907-8, **28**, 708).

Nitrogen may be detected by heating the substance (if a liquid, absorbed by asbestos or sand) with metallic sodium in a narrow test-tube. Cyanide is formed, and may be dissolved out with cold water. The filtered liquid should be treated with a drop each of ferrous-sulphate and ferric-chloride solutions, and then acidified with hydrochloric acid, when a deep green colouration or Prussian-blue precipitate will indicate that a cyanide was formed.

Most organic compounds give off the whole of their nitrogen in the form of ammonia on ignition with soda-lime. If rich in nitrogen, an addition of sugar should be made to the soda-lime, on each side of the substance to be analysed, so as to expel the air as completely as possible.

Some nitrogenised bodies, such as indigo, yield volatile organic bases, instead of ammonia, on ignition with soda-lime. These all resemble ammonia in the fact that their hydrochlorides form double salts with platinum chloride, which on ignition leave 194.8 parts of platinum for 28 of nitrogen.

Nitro-substitution compounds, such as picric acid, do not evolve the whole of their contained nitrogen in the form of ammonia when ignited with soda-lime. Addition of sugar improves the result.

Cyanogen compounds may be analysed by ignition with soda-lime if a high temperature be ultimately employed. The use of sugar is desirable.

A general process for the determination of nitrogen in organic bodies consists in combustion with oxide of copper, passing the gaseous products over red-hot metallic copper or silver, absorption of the carbon dioxide and water by solution of alkali, and measurement of the residual gaseous nitrogen. For details, see (for example) Gattermann's *Praxis des Organischen Chemikers* (7th Ed., Leipzig). (*Practical Methods of Organic Chemistry, Macmillan.*) V. Meyer found that in the case of nitrogenous bodies containing much sulphur it is neces-

sary to replace the oxide of copper by a thick layer of lead chromate, and to conduct the combustion very slowly. The nitrogen obtained should be tested for carbon monoxide. In the case of compounds containing methoxyl and ethoxyl groups the nitrogen evolved may contain large quantities of methane (see Haas, *Proc. Chem. Soc.*, 1906, 22, 81; *Trans.*, 1906, 89, 570).

Kjeldahl Method.—For routine work in organic analysis, the Kjeldahl method is now generally used. The original method employed special oxidising agents, but in most cases Gunning's modification is used. The reagents and procedure in the standard Kjeldahl-Gunning method are as follows:

Potassium Sulphate.—A coarsely powdered form free from nitrates and chlorides should be selected.

Sulphuric Acid.—This should have a sp. gr. 1.84 and be free from nitrogen compounds.

Standard Acid.—N/2 sulphuric or hydrochloric acid, the strength of which has been accurately determined.

Standard Alkali.—N/10 ammonium hydroxide, sodium hydroxide, or barium hydroxide, the strength of which in relation to the standard acid must be accurately determined.

Strong Sodium Hydroxide Solution.—Five hundred grm. should be added to 500 c.c. of water, the mixture allowed to stand until the undissolved matter settles, the clear liquor decanted and kept in a stoppered bottle. It will be an advantage to determine approximately the quantity of this solution required to neutralise 20 c.c. of the strong sulphuric acid.

Indicator.—Cochineal solution is recommended by the A. O. A. C., but methyl-orange, azolitmin, and sodium alizarinmonosulphonate are satisfactory. Phenolphthalein is not well adapted to titration of ammonium compounds.

Combined Digestion and Distillation Flasks.—Jena-glass round-bottomed flasks with a bulb 12.5 cm. long and 9 cm. in diameter, the neck cylindrical, 15 cm. long and 3 cm. in diameter, flared slightly at the mouth.

Process.—From 0.7 to 3.5 grm., according to the proportion of nitrogen, are placed in a digestion flask. Then 10 grm. of powdered potassium sulphate and 15 to 25 c.c. (ordinarily about 20 c.c.) of the strong sulphuric acid are added and the digestion conducted as follows: The flask is placed in an inclined position and heated below the

b. p. of the acid during from 5 to 15 minutes, or until frothing has
ceased. Excessive frothing may be prevented by the addition of a
small piece of paraffin. The heat is raised until the acid boils briskly.
A small, short-stemmed funnel may be placed in the mouth of the
flask to restrict the circulation of air. No further attention is required
until the liquid has become clear and colourless or not deeper than a
pale straw-colour.

When Kjeldahl operations are carried out in limited number, the
arrangement shown in Fig. 36 is satisfactory. A double-Y, terra-
cotta drain-pipe, about 20 cm. internal diameter, is connected by an
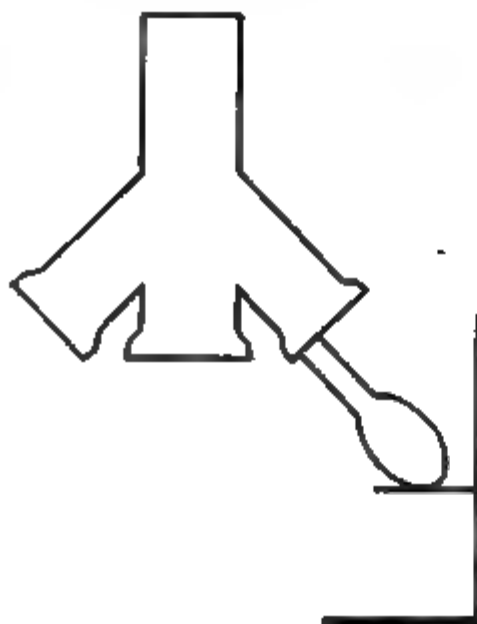
FIG. 36.   FIG. 37.

elbow directly with the chimney-stack. The digestion flasks are sup-
ported as shown in a rough sketch (not drawn exactly to scale). Two
flasks can be operated at once. The central opening is convenient
for other operations producing fumes. Openings not in use are closed
by circles of heavy asbestos.

The apparatus shown in Fig. 37 is used when many determinations
are made. As corrosive vapours are given off, it must be placed under
a hood. The central opening in the ventilating pipe shown in Fig.
36 will be satisfactory; the mouths of the flasks should be well inside
the margin of the pipe.

When the liquid has become colourless or very light straw-yellow, it is
allowed to cool, and diluted by the cautious addition of 200 c.c. of water.
Granulated zinc, pumice-stone, or 0.5 grm. of zinc dust is added.

50 c.c. of the strong sodium hydroxide solution, or sufficient to make
the reaction strongly alkaline, should be slowly poured down the side
of the flask so as not to mix at once with the acid solution.   It is con-
venient to add to the acid liquid a few drops of the indicator solution,
to show when the liquid is alkaline, but it must be noted that strong
alkaline solutions destroy some indicators.   The flask is shaken so as
to mix the alkaline and acid liquids and at once attached to the con-
densing apparatus.   The receiving flask should have been previously
charged with a carefully measured volume of the $N/2$ acid (10 c.c. di-
luted with water to 100 c.c. is a convenient amount).   A few drops of
the indicator solution should also be added.   The distillation is con-
ducted until about 150 c.c. have passed over.   The acid is then
titrated with standard alkali, and the amount neutralised by the dis-
tilled ammonia determined by subtrac-
tion.   Each c.c. of $N/2$ acid neutralised
is equivalent to 0.007 nitrogen.

The distillation in this operation re-
quires care, as the amount of ammonia
formed is determined by its neutralising
power, hence solution by the alkali of
the glass will introduce error.   Common
glass is not satisfactory.   Block-tin is
the best material for the Kjeldahl-
Gunning form, but Moerrs has shown
that it is not adapted to the methods in
which mercury oxide is employed.   He
found that Jena-glass tubes resist the
action of the alkali.   The distillates
should be titrated promptly; on standing
for some hours some alkali may be
taken up from the flask.

FIG. 38.

The most satisfactory condensing arrangement for general laboratory
use is a copper tank of good size, through which several condensing
tubes pass.   Such an arrangement as applied to Kjeldahl distil-
lations is shown in Fig. 38, which is a rough sketch, not drawn to
scale.   The flask is the standard Jena-glass distilling flask, about 12
cm. diameter, the tank should be high enough to allow of a condensing
tube 60 cm. long.   The connection of this with the receiving flask is
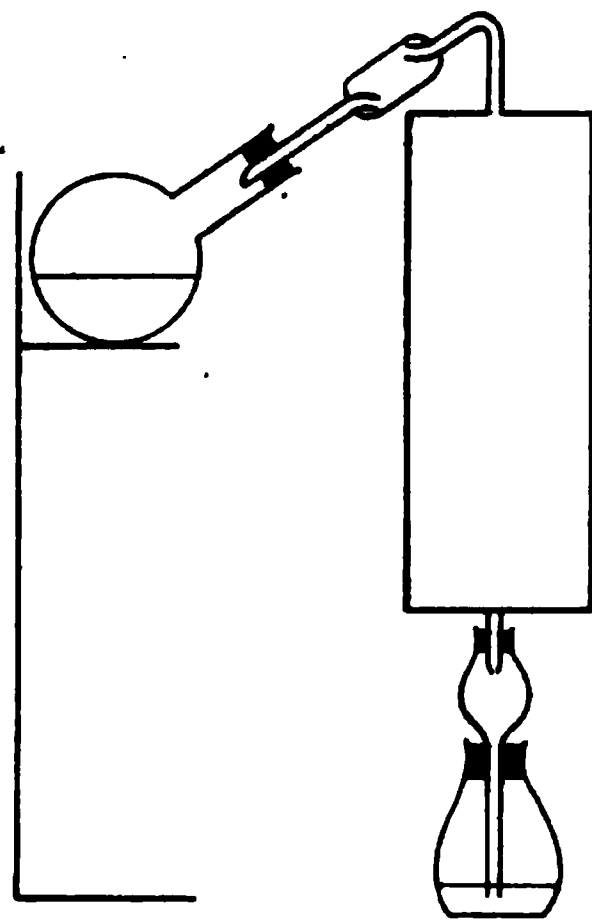made by means of a bulb tube to allow for occasional drawing back

of the liquid. The cork through which this tube passes into the flask must not fit closely, as opportunity must be given for expansion of the air. The safety-tube connecting the distilling flask with the condenser should terminate a little below the water level in the tank. The apparatus may be satisfactorily heated by a low-temperature burner. To avoid spurting of the boiling liquid, it is usual to interpose a safety tube between the distilling flask and the condenser. Many forms have been suggested. That shown in Fig. 39 is most in use.
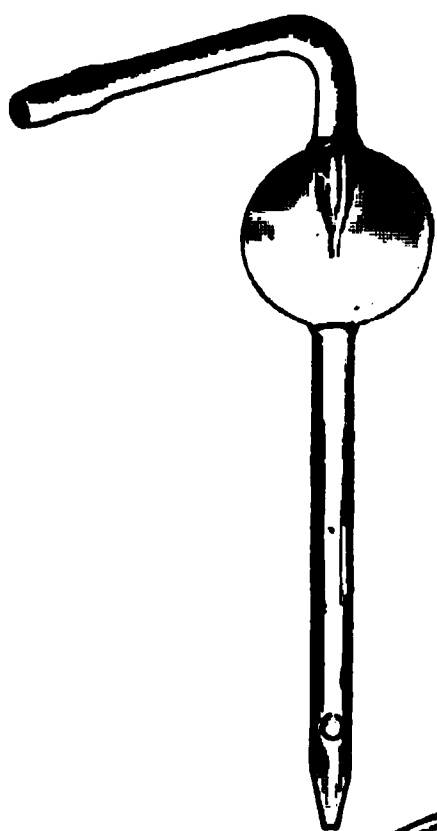
In some analyses (as in the case of pepper) the Kjeldahl-Gunning method must be replaced by Arnold's modification: One grm. of the sample is mixed with 1 grm. of crystallised copper sulphate and 1 grm. of mercuric oxide. The potassium sulphate-sulphuric acid mixture as given above is added and the mass heated cautiously until frothing ceases, when the temperature is raised and the digestion completed. The liquid is diluted for distillation, 50 c.c. of a solution of commercial potassium sulphide (40 grm. to 1000 c.c.) are added, and sufficient sodium hydroxide as usual. The liquid is liable to bump.

Modification for Nitrates.—If nitrates are

FIG. 39.

present in the material, the weighed sample is well mixed with 35 c.c. of sulphuric acid containing 2%, by weight, of salicylic acid, and the mass shaken frequently during 10 minutes; 5 grm. of sodium thiosulphate are added and 10 grms. of potassium sulphate. The mixture is heated very gently until frothing ceases and then according to the usual method. The nitrogen in the distillate will include that derived from the nitrogen of the nitrates.

Chlorine, bromine and iodine are detected in an organic substance by heating it with metallic sodium in a small glass tube, dropping the tube, while hot, into distilled water, filtering the so ution so obtained from the fragments of glass and testing with silver nitrate after acidifying with pure nitric acid. If nitrogen is present in the compound silver cyanide is formed in the above process; in such cases it is best to heat a little of the substance with pure lime in place of the sodium in the above test and, after dissolving the product in pure dilute nitric acid, to test for the halogen in the solution. The halogens are best es-

timated by Carius' method which consists in completely oxidising the substance by heating it with fuming nitric acid (of sp. gr. 1.5) in a sealed tube containing about 1.5 times the theoretical quantity of silver nitrate. The method is described in most text-books of practical organic chemistry (*e. g.*, Gattermann's *Practical Methods of Organic Chemistry*) and is very accurate. In most cases the decomposition of the compound is complete after heating 6 hours at 200–205° C.; but it is safest to heat the tube, after releasing the pressure, during another 6 hours at 250–300°, so as to insure complete decomposition.

Plimpton and Groves determine the halogens in volatile organic bodies by burning the substance gradually in a bunsen flame, placed under a trumpet-shaped tube, and absorbing the products of combustion in solution of sodium hydroxide which is subsequently acidified with nitric acid and precipitated by silver nitrate. The test analyses by this method are highly satisfactory, and the process is rapid and simple.

Sulphur, Phosphorus and Arsenic may be detected by igniting the substance with pure soda-lime mixed with an oxidising agent, such as potassium chlorate, mercuric oxide, or sodium peroxide. The residue is tested for sulphates, phosphates and arsenates. The process may be made quantitative. Another method is to heat the substance in a sealed tube with nitric acid of 1.5 sp. gr. The sulphur, phosphorus and arsenic are converted, respectively, into sulphuric, phosphoric and arsenic acids. Sulphur is also easily recognised in the solution obtained by heating the substance with sodium in testing for a halogen; it is only necessary to put a drop of the solution on a silver coin when, if sulphur is present, the coin is blackened owing to the formation of silver sulphide.

Sulphur, phosphorus and arsenic are readily estimated by oxidising the substance with nitric acid, as in Carius' method, this treatment giving rise to sulphuric acid, phosphoric acid and arsenic acid, respectively. (See page 146.)

Metals usually remain in the residue obtained on igniting the organic substance in the air. Metals of the alkalies and alkaline earths are usually left as carbonates, but when sulphonic groups are present sulphates are formed; phosphates or haloids may be formed when phosphorus or halogens are present. Heavy metals are usually left as oxides, except silver, gold and platinum, which will remain in the free

state. Arsenic, antimony and other metals, when existing in volatile compounds, may be completely volatilised. (See page 74.)

Mercury will usually be wholly volatilised. It may be estimated in all instances by igniting the substance with soda-lime, and collecting and weighing the mercury which distils over.

Oxygen may be detected by ignition of the substance in a stream of hydrogen, when water will be formed. By igniting the substance in a stream of chlorine, or in admixture with potassium chloroplatinate, carbon dioxide will be formed if oxygen be present. Hydrochloric acid and chlorine may be respectively absorbed by solutions of lead nitrate and stannous chloride, and the carbon dioxide passed into baryta water or potassium-hydroxide solution. In the great majority of cases the oxygen of organic bodies is *determined* "by difference."

MOISTURE, CRUDE FIBRE AND ASH.

These are data of *proximate analysis*, frequently required in connection with commercial organic analysis.

Moisture may be either hygroscopic or molecular. The former is always influenced by atmospheric conditions, the latter not usually. In most cases in commercial organic analysis, the hygroscopic moisture is alone important. In ordinary cases the operation is conducted in a water- or air-oven at atmospheric pressure, but vacuum drying ovens are now much used, and for some analyses an atmosphere of hydrogen is necessary. Soxhlet's oven, in which a solution of common salt in water is used, permits of the employment of a temperature slightly higher than 100°.

Moisture is usually estimated with sufficient accuracy, provided other volatile bodies are not present, by heating the material (solids should be finely divided) in a flat dish on the water-bath or in the water-oven until it ceases to lose weight. Flat platinum dishes from 4 to 8 cm. in diameter and 0.5 cm. high are well adapted to this work They should rest on porcelain or asbestos rings. Nickel dishes are often applicable, especially the broad shallow crucible covers made in dish form. Dishes of glass—especially the shallow (petri) dishes used for microbe culture—and porcelain are suitable; aluminum and tin less so. The drying of a liquid will be facilitated by using an absorbent material, such as pure quartz sand, powdered asbestos or pumice-stone. These materials should be extracted with dilute hydrochloric acid, well washed and

well dried before use. The quantity used should be rapidly weighed, preferably in the dish in which the operation is to be carried out. It is advisable to cover the dish with a nearly flat, thin watch-glass in all the weighings. By a few trials a glass can be selected which fits fairly close to the rim of the dish and restricts evaporation or absorption of water. It is often convenient to weigh a small stirring-rod with the dish and absorbent.
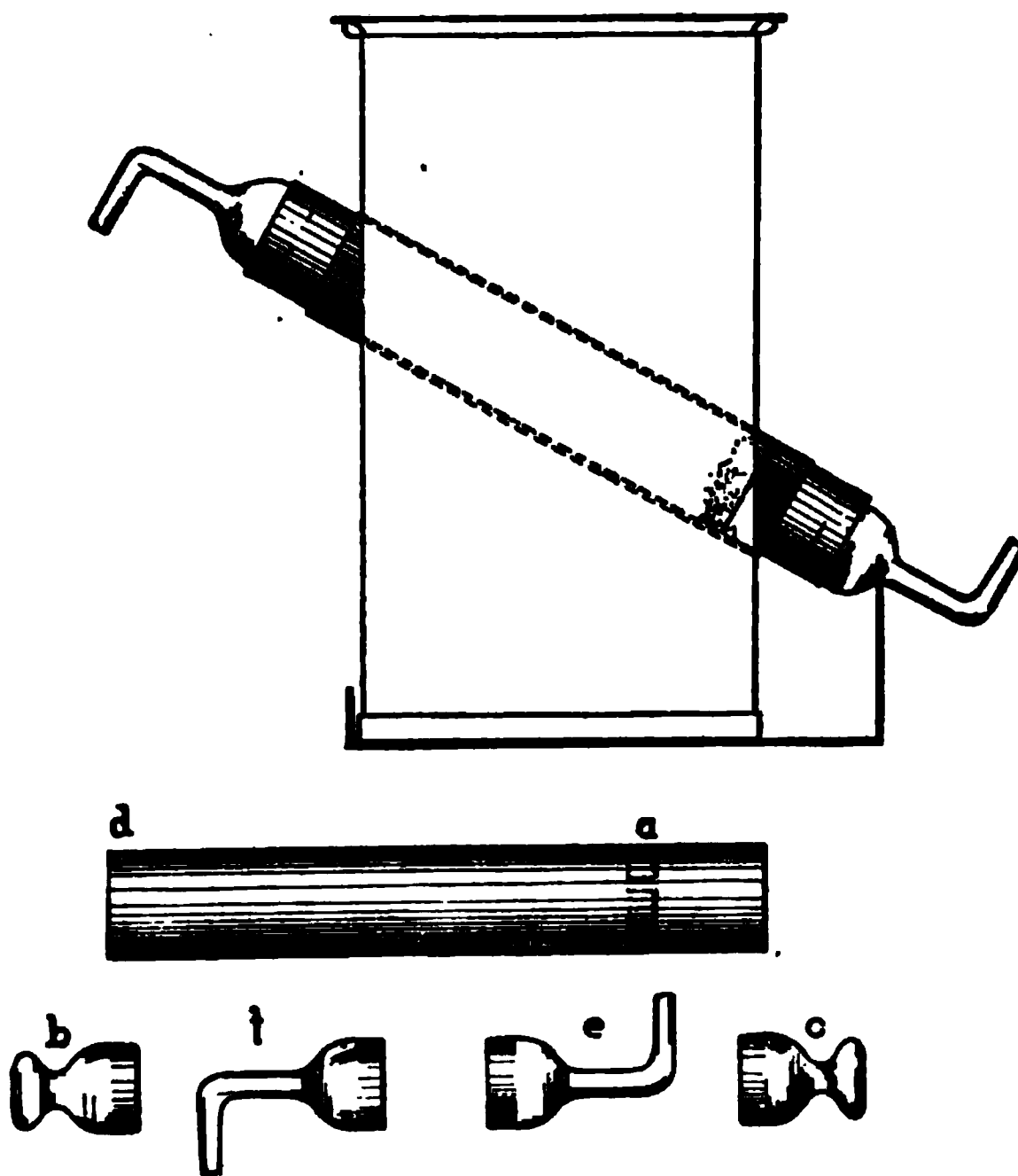
FIG. 40.

In many cases a liquid can be measured directly into the dish, the residue being recorded in grm. per 100 c.c. or other suitable ratio.

Syrupy and gelatinous liquids or those containing much solid matter, especially if this be somewhat difficult to dry, may often be more satisfactorily treated by diluting a weighed portion with several times its weight of water, evaporating a measured or weighed amount of the dilute liquid, and calculating the amount of residue in the original substance.

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The ordinary water-bath and water-oven need no description. The temperature of materials heated on the former is usually much less than 100°; in the latter, slightly below 100°.

The following are some convenient special forms of drying oven:

Fig. 40 shows a drying oven for use with a current of hydrogen. The apparatus was designed by Caldwell for estimatning moisture, ether-extract, and crude fibre as prescribed by the A. O. A. C., the three data being determined on the same sample.

The bath is made of copper and is 24 cm. long, 15 high, and 8.5 broad. It stands in a piece of sheet-copper bent at right angles along the sides, as shown in the end view; on one side this vertical part need not be over 1 cm. high, just enough to project a little up the side of the bath, which rests snugly against it; along the other side it projects upwards, at a little distance from the side of the bath, about 15 mm., and to about the height of 4 cm.; opposite each of the tubes of the bath a slot is cut in this vertical part, which serves then as a shoulder against which the glass tube rests when in place, to keep it from slipping down and out of position.

The tube for containing the substance has at the zone *a* three small projections on the inner surface, which support a perforated platinum disc of rather heavy platinum foil carrying the asbestos filter. This tube is 13 cm. long and 23 mm. inner diameter, and weighs, with its closed stoppers, about 30 grm.

The filter is readily made in the same manner as the gooch filter, the tube being first fitted to a suction flask by an enlargement of one of the holes of the rubber cork or, better still, by slipping a short piece of rubber tube over it, of such thickness that it will fit tightly in the mouth of a suction flask provided with a lateral tube for connection with the suction. A thin layer of asbestos is sufficient; if it is too thick, the gas and ether will not flow through readily.

About 2 grm. of the substance are put in this tube, previously weighed with the stoppers *b* and *c*, and the weight of the substance accurately determined by weighing tube and contents. The stoppers are removed, a band of thin asbestos paper is wound around the end *d* of the tube, a little behind the slight shoulder at the rim, as many times as may be necessary to make a snug fit, when this tube is slid down into the copper tube in the bath, thus preventing circulation of air between the glass and the copper tubes that would retard the heating of the former; the stopper *e* is put in the lower end of the tube for connection with the

hydrogen supply, and the stopper f in the upper end; this latter stopper is connected by rubber tube with a glass tube slipping easily through one of the holes of a rubber cork closing a small flask, containing a little sulphuric acid, into which this tube just dips; when as many tubes as are to be charged are thus arranged in place and the hydrogen is turned on, the even flow of the current through the whole number is secured by raising or lowering a very little the several tubes through which the outflow passes, so as to get a little more back pressure for one, or a little less for another, as may be found necessary. When the drying is supposed to be completed, the tubes are weighed again with their closed stoppers, and so on.

For ether-extraction the unstoppered tube with contents is put directly into the extractor.

Carr and Osborne have made an extended series of investigations as to the estimation of water, and find that more accurate results may be obtained if the operation be conducted under a diminished pressure at a temperature not exceeding 70°. Under these conditions it was found possible to dehydrate lævulose completely without decomposition. The oven is made of a section of metal tubing, from 15 to 20 cm. in diameter and 30 to 40 cm. long. One end is closed airtight by a brass end-piece, brazed or attached by a screw. The other end is detachable and is made air-tight by ground surfaces and a soft washer. On the top are apertures for the insertion of a vacuum-gauge and for attachment to a vacuum-apparatus, thermostat and thermometer. The aperture for admission of air or hydrogen is best placed at the fixed end. The oven may be heated by a single burner, but a series of small jets is preferable. The metal should be protected by sheet asbestos. The temperature of the oven can be kept uniform by a gas regulator or by attention to the lamp (see page 69).

The method of operating is as follows: Clean pumice-stone of two grades of fineness is used, one that just passes through a 1 mm. mesh and one that passes through a 6 mm. mesh. These are digested with hot 2% sulphuric acid, washed by decantation until the wash-water is free from acid, placed, wet, in a sand crucible and heated to redness. When the water is expelled, the material may either be placed hot into a desiccator or directly into the drying dishes. In loading the dishes, place a thin layer of dust over the bottom of the dish to prevent the material to be dried from coming in contact with the metal; over this layer place the larger particles, nearly filling the dish. If the stone has

been well washed, no harm can result from placing the dish and stone over the flame for a moment before transferring to the desiccator preparatory to weighing.

If the material to be dried is a thick liquid, it is diluted until the sp. gr. is in the neighbourhood of 1.08 by dissolving a weighed quantity in a weighed quantity of water. (Alcohol may be substituted in the case of material not precipitable thereby.) Of this, 2 to 3 grm. may be distributed over the stone in a dish the area of which is in the neighbourhood of 20 sq. cm., or 1 grm. for each 7 sq. cm. of area. The material is distributed uniformly over the pumice by means of a pipette weighing-bottle (weighing direct upon pumice will not answer), ascertaining the weight taken by difference.

The dishes are placed in the oven, which should be maintained at a pressure of not more than 125 mm. of mercury. The temperature must not exceed about 70°. All weighings must be taken with the dish covered by a close-fitting plate. The open dish must not be exposed to the air longer than absolutely necessary. Weighings may be made at intervals of from 2 to 3 hours.

In the laboratory of the United States Geological Survey a sheet-iron or nickel basin about 10 cm. in diameter and 3 cm. deep is set upon an iron plate which is heated directly by the burner. A platinum or pipe-clay triangle rests in the basin and supports the dish containing the liquid to be evaporated. It is stated that almost any liquid can be evaporated in this way without sputtering. The temperature, however, is liable to be too high for many organic bodies.

Parsons has obtained good results in the drying of sensitive organic substances by the following method: A perfectly neutral petroleum oil, free from animal or vegetable oils and mineral substances, sp. gr. 0.920, flash test 224°, fire test 260°, b. p. about 288°, is heated to about 120° for some time and preserved in a well-stoppered vessel. A quantity of oil about 6 times that of the weight of the substance to be dried is heated in an evaporating dish in a drying oven to a temperature of 115°, and then weighed. The weighed portion of the substance is put into the oil; if it be very moist, it is added in small portions. Slight effervescence will usually occur, and the mass should be kept in the drying oven for a short time after effervescence has ceased. The evaporating dish containing the oil and substance is weighed; the loss is moisture. The whole operation may be completed in less than half an hour.

CONSTANT TEMPERATURE OVENS.

An approximately constant temperature can be maintained in an ordinary hot-air oven heated by gas, by controlling the supply of gas by means of a suitable thermo-regulator, *e. g.*, a Reichert mercury regulator of the type shown in Fig. 41.

A vapour bath such as that devised by Victor Meyer and shown in Fig. 42, is often convenient for drying a substance at a known constant temperature; with such a bath almost any definite temperature can be maintained by choosing a suitable liquid to be vapourised in the outer jacket. For example, chloroform, petroleum, benzene, toluene, xylene, aniline, naphthalene give a series of definite temperatures on a rising scale. The same principle is applied in the Abati drying oven (Vereinigte Fabriken für Laboratoriums-bedarf) shown in Fig. 43.

A suitable solvent is kept vigorously boiling in the flask below the oven by means of the bunsen burner. With this apparatus it is very easy to change from one constant temperature to another by simply changing the flask and solvent.

FIG. 41.   FIG. 42.

Several types of ovens heated by electricity are also in use.

VACUUM DRYING OVENS.

In organic analysis it is often convenient to dry a substance in a vacuum at a lower temperature than the b. p. of the solvent impregnating the substance. A convenient form of apparatus for drying in a vacuum (Siderski, *Zeit. anal. Chem.*, 1890, 280) is shown in Fig. 45; it is made by the V. F. f. L. An arrangement made by the same firm for evaporating in a vacuum is shown in Fig. 44

FIG. 43.   FIG. 44.

Crude Fibre.—The proximate constituents included in this term are principally forms of cellulose, but the method given herewith, which is that of the A. O. A. C. yields a residue containing notable amounts of other substances:

2 grm. of the substance, well extracted with ether (see under "Extraction"), are mixed in a 500 c.c. flask with 200 c.c. of boiling water containing 1.25% of sulphuric acid; the flask is connected with an inverted condenser, the tube of which passes only a short distance below the rubber stopper of the flask. The liquid is brought to the b. p. as rapidly as possible and maintained there for 30 minutes. A blast of air conducted into the flask may serve to reduce the froth-

ing of the liquid.   The mass is filtered, washed thoroughly with
boiling water until the washings are no longer acid; the undissolved
substance rinsed back into the same flask with the aid of 200 c.c. of
boiling water containing 1.25% of sodium hydroxide nearly free from
sodium carbonate; again brought to the b. p. rapidly and maintained
there for 30 minutes as directed above

FIG. 45.

The liquid is filtered by means of a gooch crucible; washed with
boiling water until the washings are neutral to phenolphthalein; dried
at 110°; weighed and then incinerated completely and again weighed.
The loss of weight is crude fibre.

The filters used for the first filtration may be linen, glass, wool, asbes-
tos, or any form that secures clear and reasonably rapid filtration.   Har-
dened-paper filters may serve.   The sulphuric acid and sodium hydrox-

ide must be made up of the specified strength, determined by titration. The material must be ground very fine and the preliminary extraction with ether must not be omitted. It is probable that carbon tetrachloride could be advantageously substituted for ether in the preliminary extraction.

Crude fibre should not be called cellulose.

Ash.—The method of detecting and estimating the mineral constituents of an organic substance usually consists simply in igniting a known weight of the body in free contact with the air, and weighing the residue or *ash*.

The most satisfactory method of estimating the *ash* of organic substances is to conduct the ignition in a platinum tray or flat capsule placed in a gas-muffle maintained at the lowest temperature compatible with combustion. The tray should be supported on a row of pieces of tobacco-pipe stem or other non-conducting substance, so as to avoid over-heating from contact with the bottom of the muffle. If a bunsen burner is employed to effect combustion, similar care should be taken to avoid over-heating. If too high a temperature is employed there is great danger of loss by volatilisation of chlorides or carbonates and additional trouble may arise from fusion of the remaining ash, with consequent enclosure of particles of unburnt carbon. By keeping the temperature as low as possible, and avoiding local heating, nearly all organic substances can be burnt completely and without difficulty. In obstinate cases, the unconsumed matter may be mixed with ammonium nitrate or moistened with a strong solution of the salt and then reignited. Addition of pure mercuric oxide is also useful occasionally, or the refractory matter may be mixed with a known weight of dry ferric oxide and again ignited.

In very many instances the difficulty of effecting complete combustion and the danger of loss by volatilisation may be wholly overcome by moistening the substance to be ignited, or the carbonaceous residue therefrom, with strong sulphuric acid. This converts the readily fusible and volatile chlorides and carbonates into the more fixed *sulphates* of the alkali metals, and on ignition complete combustion will readily ensue. This method is used in ascertaining the percentage of metal in sulphonates and some other salts. It is necessary to moisten the ash with a drop of sulphuric acid and reignite, so as to get rid of any sulphides left after the first ignition. For obtaining the ash of animal matters, it is desirable to treat the substance in a porcelain

crucible with a mixture of strong nitric and sulphuric acids. This dissolves and destroys the organic matters before ignition, and, on evaporating the liquid to dryness and igniting the residue, complete combustion ensues, and a white "sulphated ash" is readily obtained. The same modification of the usual method of determining the ash of plants may be pursued with advantage in many cases, the starch and cellulose being first converted into oxalic acid, which the sulphuric acid decomposes into carbon oxides and water, so that after evaporation of the acid there is but little organic matter left to ignite.

Sulphuric acid is almost always employed in determining the ash of commercial sugars, a deduction being made from the weight obtained for the increase due to "sulphation."

Besides being in excess of the true ash, the "sulphated ash" will contain no chlorides or carbonates. Phosphoric and silicic acids are not affected by the treatment.

The estimation of ash may be facilitated by igniting the charred residue in a current of oxygen. The complete combustion of the carbon is frequently prevented by the formation of a glaze of fused mineral matter. In many cases this difficulty may be avoided by allowing the charred mass to cool, washing it with distilled water and collecting the washing through a small, nearly ashless filter; the washed residue is then burned white, the watery solution added, and evaporated to dryness.

It must be remembered, however, that carbonate in the ash is usually the skeleton of the salts of organic acids present in the original substance. Many analysts deduct the carbon dioxide in the ash from the total weight obtained, and report the difference as "true ash." A similar correction is often made for the "sand and carbon" left on treating the ash with dilute acid, the sand being merely an accidental impurity and not a true constituent of the plant, and the carbon being simply due to incomplete combustion of the organic matter.

The ordinary constituents of the ash of natural organic substances are potassium, sodium, calcium, magnesium, manganese and iron which exist as oxides, carbonates, sulphates, phosphates, silicates and chlorides. Traces of other elements exist normally in certain cases, but the foregoing are those to which attention is generally directed. In algae notable traces of bromides and iodides occur, while some other cryptogams contain aluminum. Common flowering plants and animal tissues used for human food do not contain appreciable amounts of

aluminum, but clay being a common ingredient of soils, aluminum compounds may be present as adventitious material. Copper is widely distributed, occurring in minute amount in wheat flour and some of the viscera (*e. g.*, liver) of domestic animals. It is a constant ingredient of the circulating fluid of the lobster. Lately, barium has been found as a notable ingredient in the ash of some plants from the cattle feeding districts of the western United States. It has also been found in Egyptian wheat. Zinc is present in a few rare cases.

Analysis of Ash. — Ash analysis may be effected by the ordinary methods of mineral analysis, but it should be borne in mind that the ash of wheat and other cereals is apt to contain pyrophosphates and these must be converted into orthophosphates by fusing the ash with alkaline carbonate before the ordinary process for phosphoric acid can be employed. Titration of chlorine by silver nitrate solution (with potassium chromate as indicator) cannot be effected with accuracy, unless the phosphates have been previously removed by precipitating the aqueous solution of the ash with calcium nitrate.

In many cases it is of service to ascertain the proportion of the total ash which is soluble in water. This is most conveniently done by igniting and weighing the insoluble matter and deducting the weigh-found from that of the total ash previously determined. The aqueous solution can then be used for the determination of the chlorides, alkalinity and other data. Some analysts apply the term "soluble ash" to the ash left on igniting the residue obtained by evaporating to dryness the filtered aqueous solution of the substance. This is not identical with the soluble portion of the ash of the whole substance, and should be called in preference the "ash of the aqueous extract."

The *alkalinity*, or capacity of the ash for neutralising acid, is a useful indication. It is commonly expressed in terms of K_2O, and is estimated by titrating the filtered aqueous solution of the ash with standard acid.

Poisonous metals are apt to occur as impurities in certain commercial organic products, being accidentally introduced during the process of preparation. The objectionable metals most commonly occurring are arsenic, lead, copper, zinc and tin, and in ordinary cases the search may be limited to these.

Liquids.—In some cases, as, for instance, vinegar and lemonade, the metallic impurities may be sought for in the original liquid, but in others it is desirable to evaporate the liquid carefully to dryness, ignite the residue, and test the resultant ash. The evaporation should be

conducted in porcelain. One hundred c.c. of such liquids as beer, cider or vinegar will usually suffice for the examination, but sometimes the use of considerably larger volumes is desirable. Towards the end of the evaporation, an addition of strong nitric and sulphuric acids should be made, the quantity used depending on the amount of organic matter to be destroyed. The evaporation is then carefully completed and the residue ignited at a low red heat. After cooling, the ash is moistened with nitric acid and 1 drop of sulphuric acid, and again ignited. It is then again treated with a few drops of nitric acid, which is evaporated off cautiously, the process being stopped directly acid fumes cease to be copiously evolved. The residue is then treated with hot water, and the solution filtered, when the following scheme of analysis should be followed.

Aqueous Solution may contain copper, zinc, iron. Add excess of ammonia and filter			Residue may contain lead, tin. Wash, and pour boiling solution of ammonium acetate on the filter	
Precipitate may contain iron, phosphates.	Filtrate, if blue, contains copper. Divide into two portions.		Solution Acidify with acetic acid, and add potassium chromate. A chrome yellow precipitate indicates *lead*.	Residue. Ignite filter paper, fuse ash in porcelain crucible with potassium cyanide, dissolve product in water, filter, boil insoluble residue with strong hydrochloric acid; dilute, and treat clear solution with mercuric chloride. A white silky precipitate of mercurous chloride is due to *tin*.
	1. Acidify with acetic acid and add potassium ferrocyanide. Brownish precipitate or colouration is indicative of *copper*.	2. Heat to boiling, and add potassium ferrocyanide. White precipitate or turbidity indicates *zinc*.		

Minute traces of copper are perhaps best detected by introducing a knitting needle into the slightly acidified and tolerably concentrated aqueous solution of the ash, removing it after it some hours, cautiously rinsing it in water, and then immersing it in dilute ammonia, with free contact of air. The copper precipitated on the iron will pass into solution, and may be detected by acidifying the ammoniacal liquid with acetic acid and adding potassium ferrocyanide, when a purple or brownish colouration will be produced if a trace of copper be present.

Solids may be examined for traces of the foregoing poisonous metals in precisely the same way as liquids which have been concentrated to a small bulk by evaporation.

The detection of zinc and copper in food articles has become of considerable importance lately, in view of the use of colouring matters containing these substances, and the tendency to restrictive legislation con-

cerning such use.   Much attention, for example, has been given to the
detection of zinc in dried apples, in consequence of the efforts of the
German government to prohibit the importation of American dried
apples, under the allegation that they were dangerously contaminated
with zinc derived from the plates on which the drying is conducted.
Wiley, in a bulletin published by the United States Department of
Agriculture, has given the results of an investigation into this question;
in some cases he obtained results differing materially from those
obtained upon the same samples by the German chemists.

In most cases, especially in examining food and household articles,
an amount of arsenic sufficient to be of sanitary significance may be
detected by Reinsch's test, using a liberal allowance of hydrochloric
acid, since the more highly oxidised forms of arsenic (arsenates) do
not give the reaction in the presence of small amounts of hydrochloric
acid.   Reinsch's test cannot be applied in the presence of active oxid-
ising agents, such as chromates, chlorates or nitrates.   Processes for
examination of beer, glucose and foods for arsenic will be described
in the sections devoted to such substances.

The examination for arsenic and poisonous metals in cases of sus-
pected poisoning does not come within the scope of this work and will
not be described.

The detection of alum and other mineral adulterants of flour and
bread is described under "cereals."

BEHAVIOUR OF ORGANIC SUBSTANCES WITH SOLVENTS.

In the proximate analysis of plants and other complex substances
of organic origin, a systematic treatment with solvents is a most valu-
able means of separating different classes of compounds from each
other·   The systematic use of solvents has been worked out very
thoroughly by Dragendorff and others, whose methods will be described
in greater detail in future sections.   In proximate organic analysis
only a limited use is made of the stronger acids so largely employed
in mineral analysis, while the use of alcohol, ether, chloroform and
other organic solvents is greatly extended.

Exhaustion of Organised Tissues by Solvents.—In assay-
ing commercial organic substances it is often requisite to effect as
perfect an exhaustion as possible of an organised tissue of some active

principle or valuable constituent existent therein. This is the case in the assay of cinchona barks for alkaloid, of seeds and oil-cakes for oil, and of sugar-cane and beet-root for sugar. In such cases the cells which contain the principles to be extracted are only incompletely ruptured by the most careful pounding or crushing of the sample, and hence solvents can only act on the contents through the cell walls, and the resultant solution can only pass through the cell walls by diffusion. This often renders the process of exhausting organised tissues very tedious, while the difficulty is enhanced by the fact that economy and convenience of subsequent treatment often render it desirable or necessary to use a very limited quantity of solvent. Under these circumstances, an apparatus which will act almost automatically and allow of complete exhaustion by a small quantity of solvent possesses great advantages.

Soxhlet's Tube.—For the automatic exhaustion of a substance by a volatile solvent, no better arrangement has been described than an ingenious device of Szombathy, commonly called Soxhlet's apparatus (Fig. 46). The substance to be extracted is inclosed in a plaited filter or cylinder of filter-paper, or if it be coarse it is sufficient simply to place it loose in a large test-tube, having an aperture at the bottom closed by a plug of glass-wool. Thus arranged, the tube or filter with its contents is placed in a Soxhlet tube, having a little glass-wool at the bottom, and adapted by means of a cork to a flask containing the solvent. A vertical condenser is adapted to the upper end of the Soxhlet's tube, and the solvent kept boiling by a suitable source of heat. In the case of petroleum spirit, ether or other volatile and inflammable solvent, this should be a water-bath kept hot by a small flame or an electric stove. As the solvent boils it is condensed and falls on the substance to be extracted, remaining in contact with
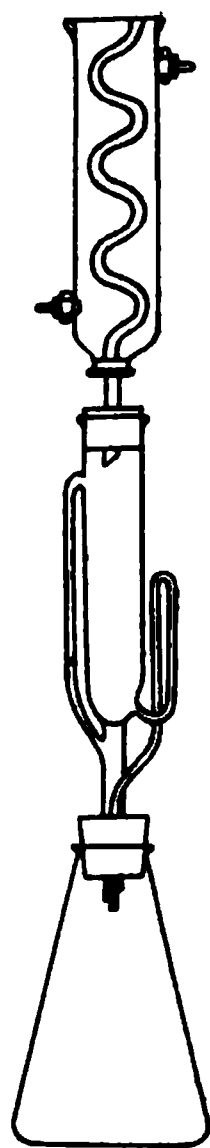
FIG. 46.

it until both the inner and outer tubes are filled to the level of the syphon, when the solution passes off into the flask, to be redistilled and recondensed, and so on until the process is judged to be complete. With a proper arrangement of the source of heat, the extraction goes on regularly and automatically. On changing the flask and replacing the inner tube by one containing a fresh sample, the apparatus is ready to be used for another extraction.

The paper thimbles made by Schleicher and Schüll are convenient for use with extraction apparatus.

A very simple and convenient form of exhauster, adapted either for extraction or repercolation, has been described by Dunstan and Short (*Pharm. Jour.*, 1882–3, [3], **13**, 664). It consists of two glass tubes, the wider of which is drawn out at one end. The narrower and somewhat shorter tube fits into the outer one with much margin, and is also drawn out in such a way as to allow the end to protrude from the drawn-out end of the wider tube when the smaller is inserted therein. At the point where the outer tube commences to contract it is indented on opposite sides, by which means two ledges are formed within the tube which serve as supports for the narrower tube.[1] The inner tube serves to contain the substance to be exhausted. The lower drawn-out end of the wider tube is .fitted by a cork to the flask containing the volatile solvent, while the upper end is connected with a condensing arrangement.

J. West-Knights has described a form of exhauster which may be conveniently used when the quantity of material to be extracted is somewhat small (*Analyst*, 1883, **8**, 65). A percolator is made by cutting off the bottom from a test-tube of suitable size, and blowing a hole in the side of the tube about an inch from the top. A disc of filter-paper or fine cambric is tied over the lower end of the tube. The substance to be extracted is placed in the tube, and kept in its place by some glass-wool and a perforated disc of metal, and the tube with its contents then fixed by a cork to the lower end of the tube of a vertical condenser. This is adapted by a larger cork to the neck of an ordinary flask containing the volatile solvent, on heating which the vapour passes through the hole in the side of the test-tube up into the tube of the condenser, where it is liquefied. The condensed liquid drops right into the test-tube, percolates through the substance to be extracted, and falls to the bottom of the flask, to be again volatilised. As the percolator is inside the flask, its contents are kept constantly at the b. p. of the solvent, and, the action being continuous and automatic, very rapid exhaustion may be effected.

The "flow" extractor devised by Burgess and shown in the sketches in Figs. 47 to 49 (apparatus made by Müller, Orme and Co., London) is more effective than the Soxhlet for many purposes, the substance

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[1] The indentations are made by gently pressing each side of the ̣tube when red-hot with a pair of crucible tongs.

being kept covered with the solvent in use and a flow of clean solvent continuously passing through it. The joints are ground-in joints.

Other forms of exhauster have been contrived by Church, Drechsel, Angell, Thoms, Thresh (*Pharm. Jour.*, 1884-5, [3], 15, 281) and others, but those described will be found sufficient for most purposes.

Employment of Immiscible Solvents.—In mineral analysis this method finds but few applications, but in proximate organic anal-
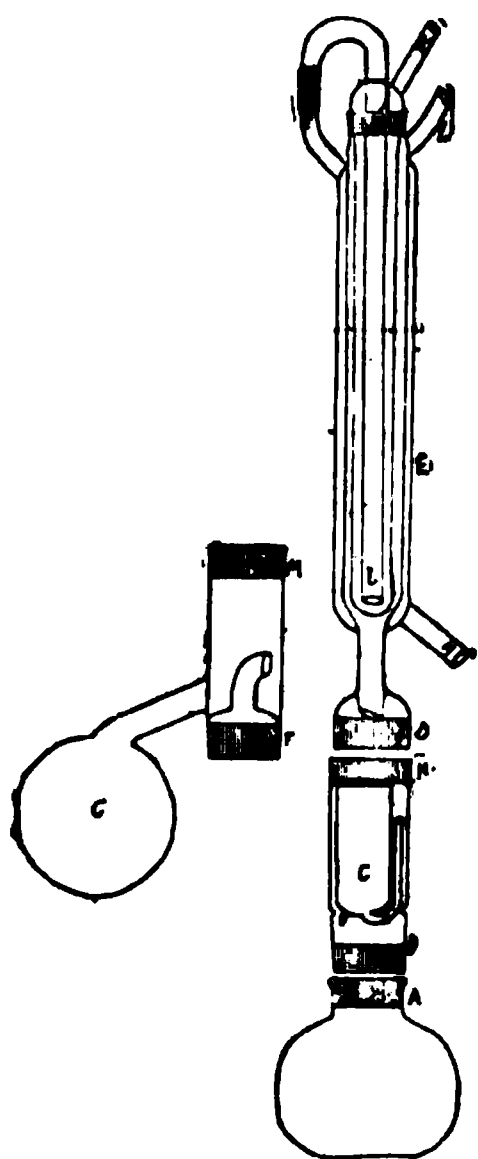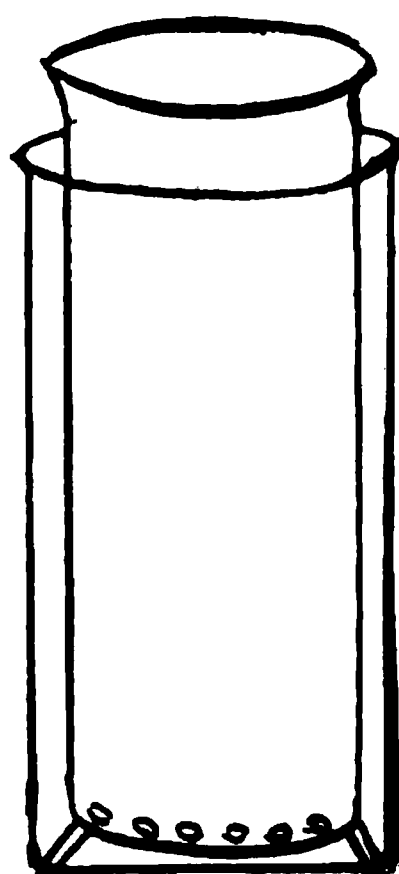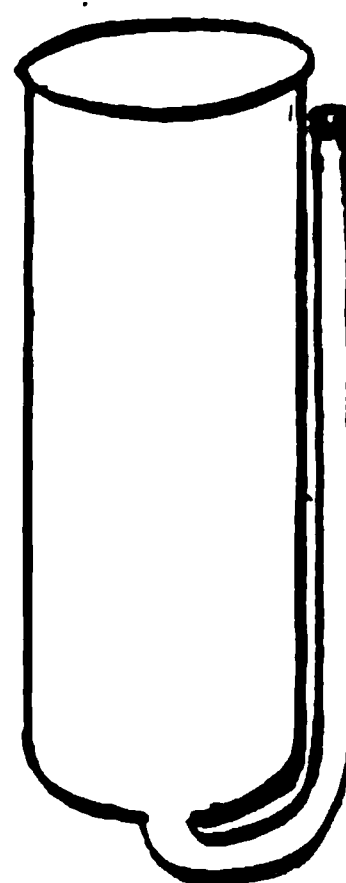
FIG. 47.   FIG. 48.   FIG. 49.

ysis one of the most valuable means of effecting separations consists in agitating the solution of a substance in one solvent, with another solvent insoluble or only slightly soluble in the former liquid. Under these circumstances, the dissolved body is distributed between the two solvents in proportions which are dependent on the relative solubility of the substance in the two media, and the relative quantities of the two media employed. Thus, it may be supposed that, if a substance be 99 times more soluble in chloroform than in water, and its aqueous solution be shaken with an equal measure of chloroform, 99% of the whole substance will pass into the chloroform. On separating this layer, and again agitating the residual aqueous liquid with an equal

quantity of chloroform, 99% of the remaining substance will be dissolved, thus making the exhaustion practically complete. The distribution of a solid between two non-miscible solvents is dealt with in works on Physical Chemistry, for example, Nernst's "Theoretische Chemie."

In making a proximate analysis by means of immiscible solvents, much of the success in practice depends on the care and skill with which the manipulation is conducted. The most convenient apparatus for effecting the treatment consists of a pear-shaped (Fig. 50) or cylindrical glass separator, furnished with a tap below and a stopper at the top. The tube below the tap should be ground obliquely so as to prevent loss of liquid by imperfect delivery. Supposing that it be desired to effect the separation of a substance from an aqueous liquid by agitation with ether, the former is introduced into the separator, of which it should not occupy more than one-third, acid or alkali added as may be desired, and next a volume of ether about equal to that of the aqueous liquid. The stopper is then inserted and the whole thoroughly shaken together for a minute or two, and then set aside. As a rule, the contents will readily separate into two well-defined layers, the lower of which is aqueous and the upper ethereal. Sometimes separation into layers does not occur readily, the liquid remaining apparently homogeneous, forming an emulsion or assuming a gelatinous consistency. In such cases separation may sometimes be induced by thoroughly cooling the contents of the separator. In the case of ether, the separation may always be effected by adding an additional quantity of ether and reagitating, or, when the employment of a sufficient excess of ether is inconvenient or impracticable, the addition of a few drops of alcohol, followed by a gentle rotatory motion of the liquid, will almost invariably cause separation to occur promptly.

Separation having taken place, the aqueous layer should be run off by the tap into another separator, where it can again be agitated with ether to insure the complete removal of the substance to be dissolved therein. The ethereal liquid remaining in the first separator should be shaken with a fresh quantity of alkaline or acidified water, which is then tapped off as before, and the remaining traces removed by

FIG. 50.

treating the ether with a little pure water. This having in turn been run off to the last drop, the ethereal solution can next be removed by the tap, but a preferable plan is to pour it out of the top of the separator, by which means any contamination by the traces of water adhering to the sides of the glass will be avoided.

When amyl alcohol, benzene, or petroleum ether is employed, the manipulation is the same as that just described; but when chloroform is used or a mixture containing a considerable proportion of that solvent, the aqueous liquid forms the upper stratum, and the chloroform solution can at once be removed by the tap.

The tendency to form an obstinate emulsion is greater when the aqueous liquid is alkaline, and is often very troublesome when chloroform, benzene, or petroleum spirit is substituted for ether. In such cases the employment of a larger quantity of the solvent sometimes causes separation, but, when admissible, a better plan is the addition of ether. This answers very successfully for the isolation of strychnine, which is nearly insoluble in unmixed ether, but readily soluble in a mixture of equal measures of ether and chloroform. This solvent is heavier than water and is capable of very extensive application.

It is evident that the treatment can be repeated any number of times requisite to ensure the complete extraction of substances having a limited solubility in the solvents employed, and these can themselves be varied in a systematic manner, as is done in Dragendorff's method for the separation of alkaloids and other active principles.

The separation of immiscible solvents is in many cases promoted by rapid rotation. The centrifugal machines employed for the rapid analysis of milk do not usually give sufficient speed for this purpose, but some of the smaller forms intended for clinical work can be operated at very high velocity, and by their use a small amount of such mixtures can often be separated rapidly and thoroughly.

Care must be taken to ascertain the purity of the solvents used in these methods, especially when toxicological investigations are being conducted. Vaughan has reported a case in which a sample of ether made by a prominent house contained a poisonous substance in such amount that the residue left by the evaporation of 50 c.c. of the ether killed a guinea-pig in a short time. Chloroform often contains corbonyl chloride, in which case it is also acid owing to the presence of hydrogen chloride.

Fig. 51 shows a special apparatus for use with solvents lighter than water.

The cylinder A should hold about 1000 c.c.   Two openings are not necessary, since both tubes may pass through the cork, but the arrangement shown is more convenient.   600 c.c. of the solution are placed in the cylinder, 300 c.c. of solvent added and the mixtures well shaken.   The rest of apparatus is then attached.   The flask B has a capacity of 200 to 300 c.c.; the solvent in it is heated by a water-bath.   The vapour passes by a into b, the condensed liquid flows to the bottom of A and rises through the solution; the upper layer returns through c into B.   The tube c should not extend into the liquid in B. A small quantity of aqueous liquid may collect at intervals in B and should be removed.

The table on page 83 shows the behaviour of the principal organic substances on treatment with water, made slightly acid or alkaline, and solvents immiscible therewith, such as ether, chloroform, amyl alcohol, benzene, and petroleum ether.   It must not, however, be supposed that the immiscible solvents can be employed indifferently, as some of the substances are removed from their aqueous solutions by one solvent, but are unaffected by others owing to their limited solubility therein. This is especially the case with the alkaloids and glucosides, and hence the table must merely be regarded as showing the general tendency, their behaviour when treated with the individual solvents being deferred for full description later.

FIG 51.

TABLE SHOWING THE BEHAVIOUR OF ORGANIC SUBSTANCES WITH IMMISCIBLE SOLVENTS.

On agitating the substance with water, acidified with sulphuric acid, and a suitable solvent immiscible therewith (such as ether, chloroform, amyl alcohol, benzene or petroleum spirit), the following distribution will occur:

ums,

lcohol,

oxalic,
ihenol.

Bases;
e, sol-
xonine,

indigo

cantharidin, picrotoxin.

products.
Proteins and their Allies; as albumin,
casein, gelatin.

ALCOHOLS.

By G. C. JONES, F.I.C. CONSULTING CHEMIST.

The term "alcohol" when used without qualification and as a proper name is generally, and throughout this book always, to be understood as applying to ethyl alcohol.

This section will deal only with those monohydric aliphatic alcohols which are either themselves of commercial importance or which are essential constituents or commonly occurring impurities of other articles of commerce.

A slight difficulty in the arrangement of the matter of the section exists. In that part which deals with beer and wines the difficulty does not arise; clearly that part must deal with the analysis of beer and wines and the estimation in them of alcohol and other chemical entities. Ethyl and methyl alcohols are incapable of analysis in the commercial sense, and perhaps the analysis of commercial alcohol and wood spirit should have been relegated to that part of the section which deals with "spirits," as their analysis is largely a matter of estimating the amount of their impurities. Such an arrangement would have left under the chief headings only methods for the detection and estimation of ethyl and methyl alcohols in liquids of which they are not principal constituents. In the circumstances it has been decided to retain the arrangement of the third edition of this work as far as possible, owing to the fact that many analysts are acquainted with that arrangement. For the convenience of others the various subsections and parts of these will be somewhat more clearly indicated than in the third edition of this work.

METHYL ALCOHOL.

Carbinol, Purified Wood Spirit, $CH_3.OH$.

The chief source of methyl alcohol is the aqueous portion of the distillate which results from the dry distillation of wood, but considerable quantities are now obtained from *vinasse*, the residue remaining after the distillation of beet molasses.

Pure methyl alcohol is a colourless, mobile liquid with only a very

faint pleasant odour. Its b. p., according to Fuchs, ranges from 65.06° at 710 mm. to 68.00° at 790 mm., and can be calculated for any intermediate pressure from these numbers (*Zeits. angew. Chem.*, 1898, 38, 871). The b. p. may be used as a test of reputed 100% methyl alcohol. The low number given by some workers, who were at great pains to dehydrate the spirit, is no doubt due to acetone which depresses it notably, a minimum being reached when the acetone amounts to 10% (Petitt, *J. Physical Chem.*, 1899, 3, 349). The melting-point, according to Ladenburg and Krugel, is —94.9° (*Ber.*, 1899, 32, 1821). The sp. gr. at 15°/15° is, according to Klason and Norlin, 0.796472 (*Arkiv Kem. Min. Geol.*, 1906, 2, No. 24, 6). These authors have recalculated the met,hyl alcohol tables of Dittmar and Fawsitt (*Trans. Roy. Soc. Edin.* 1889, 33, ii, 509), and their results occupy 26 pages in the *Arkiv Kem. Min. Geol.*, 1907, 2, No. 27. The reviser of this section has had frequent occasion to use a methyl alcohol table and has found abundant proof that the course commonly recommended, namely to break down to one-third strength and use the ethyl alcohol tables, may lead to serious errors. At 46.4% by weight the numbers in the tables are identical, but at 25 per cent. strength the error introduced by using the ethyl alcohol table for estimating methyl alcohol is more than 9%. An abridgement of Klason and Norlin's table follows.

Specific Gravity 15°/15°	Methyl Alcohol, per cent. by weight.	Specific Gravity 15°/15°	Methyl Alcohol, per cent. by weight.	Specific Gravity 15°/15°	Methyl Alcohol, per cent. by weight.
0.7965	100.00	0.814	93.74	0.832	87.24
0.797	99.82	5	.39	3	86.88
8	.47	6	.03	4	.52
9	.11	7	92.68	5	.16
0.800	98.75	8	.32	6	85.79
1	.39	9	91.96	7	.42
2	.03	0.820	.60	8	.04
3	97.67	1	.24	9	84.67
4	.31	2	90.88	0.840	.29
5	96.96	3	.52	1	83.91
6	.60	4	.16	2	.53
7	.25	5	89.80	3	.15
8	95.89	6	.43	4	82.77
9	.54	7	.07	5	.39
0.810	.18	8	88.70	6	.01
1	94.82	9	.34	7	81.63
2	.46	0.830	87.97	8	.25
3	.10	1	.61	9	80.86

Specific Gravity 15°/15°	Methyl Alcohol, per cent. by weight.	Specific Gravity 15°/15°	Methyl Alcohol, per cent. by weight.	Specific Gravity 15°/15°	Methyl Alcohol, per cent. by weight.
0.850	80.47	0.901	58.90	0.952	31.69
1	.08	2	.43	3	.07
2	79.69	3	57.96	4	30.44
3	.30	4	.49	5	29.79
4	78.91	5	.01	6	.15
5	.51	6	56.53	7	28.48
6	.11	7	.06	8	27.80
7	77.71	8	55.58	9	.12
8	.30	9	.11	0.960	26.44
9	76.90	0.910	54.64	1	25.73
0.860	.50	1	.18	2	.02
1	.10	2	53.72	3	24.31
2	75.70	3	.25	4	23.59
3	.30	4	52.79	5	22.89
4	74.89	5	.31	6	.19
5	.49	6	51.83	7	21.49
6	.09	7	.34	8	20.79
7	73.69	8	50.85	9	.09
8	.29	9	.35	0.970	19.38
9	72.89	0.920	49.84	1	18.68
0.870	.48	1	.33	2	17.98
1	.07	2	48.81	3	.28
2	71.65	3	.28	4	16.58
3	.23	4	47.74	5	15.85
4	70.81	5	.20	6	.12
5	.38	6	46.66	7	14.40
6	69.95	7	.12	8	13.67
7	.53	8	45.57	9	12.97
8	.10	9	.03	0.980	.27
9	68.68	0.930	44.49	1	11.61
0.880	.25	1	43.96	2	10.94
1	67.83	2	.42	3	.26
2	.40	3	42.88	4	9.58
3	66.97	4	.34	5	8.94
4	.53	5	41.79	6	.29
5	.09	6	.23	7	7.64
6	65.65	7	40.68	8	6.99
7	.20	8	.12	9	.36
8	64.75	9	39.56	0.990	5.72
9	.31	0.940	.00	1	.10
0.890	63.86	1	38.42	2	4.47
1	.42	2	37.84	3	3.89
2	62.98	3	.24	4	.30
3	.54	4	36.64	5	2.72
4	.09	5	.03	6	.14
5	61.65	6	35.42	7	1.62
6	.20	7	34.81	8	.10
7	60.75	8	.20	9	0.55
8	.29	9	33.58	1.000	0.00
9	59.83	0.950	32.95		
0.900	.36	1	.32		

Methyl alcohol containing only a small amount of water and a slight trace of acetone is now largely sold in the United States, usually under proprietary names, such as "Columbian Spirit," " Colonial Spirit," " Kahol." It has been much used as a substitute and adulterant for ethyl alcohol, especially in tinctures and varnishes.

Detection of Methyl Alcohol.—Only those tests which distinguish sharply between methyl and ethyl alcohols are of practical importance and only such will be dealt with here. The quickest and most satisfactory are those which depend on the oxidation of the methyl alcohol to formaldehyde by means of a heated copper wire, and the identification of the formaldehyde by a colour reaction with resorcinol. There are several modifications of this test, some quicker and less sensitive, others requiring rather more time but capable of detecting smaller amounts of methyl alcohol.

Mulliken-Scudder Test (*Amer. Chem. J.*, 1899, 21, 266).—A copper-wire spiral is heated to redness and plunged into 2 c.c. of the liquid to be tested or, if the latter is a strong spirit, into 3 c.c. of a solution diluted so as to contain not more than 20% total alcohols. The treatment with hot oxidised copper wire is repeated three or four times. One drop of 0.5% aqueous solution of resorcinol is added, and the mixture poured cautiously down the side of a test-tube containing a little concentrated sulphuric acid. A rose-red contact ring, and on gentle shaking red flocks, will appear if the original liquid contained much methyl alcohol. The test can be carried out in five minutes and shows 8 to 10% of methyl alcohol in ethyl alcohol. More sensitive reagents for formaldehyde exist, for example gallic acid, but its use is not permissible as ethyl alcohol itself, when oxidised by a copper spiral, gives enough formaldehyde to show the gallic acid reaction.

United States Pharmacopœia Test.—This is a modification of the last, occupying about fifteen minutes and showing 2% of methyl alcohol in ethyl alcohol. The liquid is diluted so as to contain about 10% total alcohols, and placed in a test-tube surrounded by cold water. It is oxidised by five or six applications of the hot copper spiral, after which it is filtered and boiled till free from any odour of acetaldehyde. After cooling, addition of one drop of 0.5 per cent. resorcinol solution and pouring on to sulphuric acid, it is allowed to stand three minutes, after the end of which time it is gently rotated. If no rose-red ring appears, less than 2 per cent. of methyl alcohol is

present. Acetaldehyde gives with resorcinol a yellowish-brown ring and flocks, hence the advantage of expelling it from the solution. It need scarcely be pointed out that the red ring and flocks are given by all substances which yield formaldehyde on oxidation, but added methyl alcohol is the only such substance likely to be present in quantity in commercial spirits or tinctures.

Test for Quantities Under 2 Per Cent.—The liquid (50 c.c.) is three times fractionated through a rod-and-disc or "pear" still-head, and the final first fraction diluted and submitted to the United States Pharmacopœia test. With strong spirit, successive first fractions of 35 and 20 c.c. may be collected and redistilled, and the first 1 c.c. of the third distillation taken for the test. If the liquid to be examined is only weakly alcoholic, a vinegar for instance, successive first fractions of 20 and 10 c.c. may be collected and the first 3 c.c. of the third distillation taken for the test. As small an amount as 0.1% of methyl alcohol may be detected in this way.

The Sanglé-Ferrière-Cuniasse Test (*Ann. Chim. anal.*, 1903, **8**, 82), which depends on oxidation by permanganate and the use of phloroglucinol as reagent, is slow, but a quicker modification by Scudder and Biggs is worth description, because when permanganate is used as oxidising agent less acetaldehyde seems to be formed than when hot copper is used. Scudder and Biggs (*J. Amer. Chem. Soc.*, 1906, **28**, 1202) recommend that to 10 c.c. of the solution to be tested, 0.5 c.c. of concentrated sulphuric acid and 5 c.c. of a saturated solution of potassium permanganate be added. The temperature should be between 20° and 25°: below 18° action is slow, while above 30° formaldehyde may be lost. After two minutes, the solution is decolourised by sulphurous acid and boiled till it no longer smells either of this or of acetaldehyde. It is then tested with resorcinol. It is important not to add much more than the stated quantity of sulphuric acid, and a control experiment with pure ethyl alcohol is advisable to those who have no great experience of the test.

Trillat Test.—As it is claimed by more than one worker that this test (*Compt. rend.*, 1899, **127**, 232) is capable of detecting 0.1% of methyl alcohol in ethyl alcohol, it must be referred to. The reviser of this section would not venture to recommend it unless several pages could be spared to describe the difficulties which surround it. Moreover, it occupies five hours, in which time the amount of methyl alcohol may be accurately estimated by the method of Thorpe and

Holmes. Those who have time to acquire skill in this very interesting but difficult test are referred to a paper by Scudder (*J. Amer. Chem. Soc.*, 1905, **27**, 892) which reviews all the principal tests which had been proposed before that date for the detection of methyl alcohol.

Voisenet (*Bull. Soc. chim.*, 1906 [iii], **35, 748**) has described a test which is stated to be sufficiently sensitive to detect one part of methyl alcohol in 20,000 of ethyl alcohol. As the method is based on the regulated oxidation of the sample by chromic acid mixture, the claim must be received with caution as ethyl alcohol itself yields appreciable traces of formaldehyde when oxidised by chromic acid mixture even in the cold.

Hinkel (*Analyst*, 1908, **33**, 417) has investigated several of the methods which depend on the oxidation of methyl alcohol to formaldehyde and the detection of this substance, and he finds morphine hydrochloride to be the most delicate reagent (see under " Formaldehyde ") for the latter purpose, and as oxidising agent prefers ammonium persulphate, which is said to produce but little formaldehyde from ethyl alcohol. To 1 c.c. of the spirit suspected to contain methyl alcohol, 0.8 grm. of ammonium persulphate and 3 c.c of dilute sulphuric acid (1:5) are added and the mixture diluted with water to 20 c.c. and distilled. The distillate is collected in test-tubes in five separate portions of 2 c.c. at a time. The first two portions, which contain all the acetaldehyde, are rejected and each of the remaining portions tested as follows: To each portion a few drops of 0.5% solution of morphine hydrochloride are added and strong sulphuric acid is poured into each test-tube so as to form a layer at the bottom. In the presence of formaldehyde, a violet ring is formed at the junction of the liquids. Acetaldehyde gives an orange colour with the reagent, as may be seen by testing the first portion of the distillate. The reagent is capable of detecting one part of formaldehyde per million parts of water, so that a control experiment with pure ethyl alcohol is absolutely necessary, since, even when ammonium persulphate is used as oxidising agent, ethyl alcohol itself gives rise to notable traces of formaldehyde. Hinkel does not claim to be able to detect with certainty less than 5% of methyl alcohol in ethyl alcohol.

For the *detection of methyl alcohol in* commercial *formalin*, in which it commonly occurs, it is of course necessary to remove the formaldehyde before applying the red-hot copper test. Many methods for effecting this removal have been suggested, and perhaps the simplest

is that of Leffmann (*Chem. Zeit.*, 1905, 29, 1086), who recommends simple distillation with a slight excess of potassium cyanide. (See p. 93.) The distillate should be tested for formaldehyde, and a second distillation is generally necessary. Other methods will readily suggest themselves to any chemist.

Estimation of Methyl Alcohol.—When nothing but methyl alcohol and water is present, the proportion of the former may be obtained from the specific gravity by reference to the tables which precede.

No general method for the estimation of methyl alcohol exists. It is only possible to describe methods which are applicable to the particular mixtures in which the analyst is most frequently called upon to estimate it. For example, in the assay of commercial wood naphtha it is usual to convert the methyl alcohol into methyl iodide and to estimate this. As all methoxy- and ethoxy-compounds yield volatile iodides under the conditions of the experiment, it is necessary to correct for the methyl acetate present in wood naphtha, and the method is clearly useless for mixtures of methyl and ethyl alcohols. On the other hand, the best method yet described for the estimation of methyl alcohol in admixture with ethyl alcohol is quite inapplicable to wood naphtha, since it depends on the oxidation of the methyl alcohol to carbon dioxide under conditions in which acetone and methyl acetate are similarly oxidised.

For the *estimation of methyl alcohol in wood naphtha* the following modification of Krell's method is adopted in the British Government Laboratory:

"22 grm. of coarsely-powdered iodine and 5 c.c. of distilled water are placed in a small flask and cooled by immersion in ice-cold water. Then 5 c.c. of the wood spirit (60.0° o.p.) are added, the flask corked, the contents gently shaken, and allowed to remain in the ice-cold bath for 10 to 15 minutes.

"When well cooled, 2 grm. of red phosphorus are added to the mixture of spirit and iodine in the flask, and the latter is immediately attached to a reflux condenser.

"The reaction soon commences, and must be moderated by dipping the flask into a cold water-bath. (Spirit may be lost if the reaction is too violent.) After about 15 to 20 minutes, when all action appears to have ceased, the water-bath under the flask is gradually heated to a temperature of about 75° (167° F.), and the flask being occasionally shaken is allowed to remain at this temperature for 15 to 20 minutes.

The source of heat is then removed and the apparatus left for an hour till it has cooled, when the condenser is reversed, and the methyl iodide slowly distilled off—first at a low temperature—the bath being allowed to boil towards the end of the operation only. The end of the condenser dips into water in a measuring tube, and the iodide is collected under water and measured at a temperature of 60° F.

"The percentage by volume is found from the formula:

$$\frac{\text{c.c. methyl iodide found} \times 0.6 \times 100}{\text{c.c. wood spirit taken}} = \text{percentage of methyl alcohol.}$$

"Or when 5 c.c. of spirit are taken:

$$\text{c.c. methyl iodide} \times 12.94 = \text{percentage by volume.}$$

"Ester, and acetals, also yield methyl iodide by this process, and from the percentage of methyl alcohol calculated as above an amount equivalent to the percentage of these substances present must be deducted. Practically, however, methyl acetate is the only compound usually found in quantity sufficient to materially affect the result. The number of grm. of methyl acetate per 100 c.c. of spirit multiplied by 0.5405 gives the equivalent of methyl alcohol to be deducted from the total percentage by volume calculated from the methyl iodide found."

The accuracy of the method is limited by the accuracy with which the 6 c.c. or so of methyl iodide can be measured. Methyl iodide is not quite insoluble in water, and it has been suggested that a correction for this solublity and for the vapour which remains in the apparatus should be made once for all by distilling 6 c.c. of pure methyl iodide and noting the deficiency from 6 c.c. of the distillate. On the other hand, some of the acetone which distils over remains dissolved in the methyl iodide; the volume so dissolved is small if the distillation be conducted as above described, but it does to some extent compensate for the loss of methyl iodide and perhaps renders unjustifiable any such refinement as the correction referred to.

Theoretically, all the sources of error enumerated above would be avoided by having recourse to Zeisel's method for the estimation of methoxy-groups, in which the whole of the methyl iodide vapour is swept out of the apparatus by a current of carbon dioxide and decomposed by alcoholic silver nitrate solution yielding silver iodide which can be weighed with any degree of accuracy desired. Stritar and Zeidler (*Zeit. anal. Chem.*, 1904, **43**, 387) have advocated this pro-

cedure, and have suggested some simplification of Zeisel's apparatus. Duplicate analyses, however, differ by 1% so that this tedious gravimetric method has little cause to be preferred on the score of accuracy to the simpler volumetric one.

The Estimation of Methyl Alcohol in Formaldehyde solutions is sometimes necessary. It may be present to the extent of nearly 20 per cent. Distillation with some reagent which forms non-volatile compounds with aldehydes and is itself non-volatile, (see page 90), will suggest itself, but there are practical difficulties. A large quantity of the reagent is required and the best, sodium phenylhydrazine sulphonate (Hewitt's reagent), is costly. Gnehm and Kaufler (*Zeit. angew. Chem.*, 1904, **17**, 673) use sodium sulphanilate, 90 grm. of which they add gradually to 25 c.c. of water which is kept boiling till all is dissolved. The flask containing the mixture is rapidly cooled, the contents being all the time stirred with a rod. To the crystalline mass 20 c.c. of the formalin is added, the flask corked and left three or four hours. The mixture is next submitted to distillation and the first 35 c.c. of the distillate collected. This is diluted to 50 c.c. and its sp. gr. taken.

The following method (Duyk, *Ann. Chim. anal.*, 1901, **6**, 407) is in use in the Paris municipal laboratory.

To 100 c.c. of the sample, diluted with 50 c.c. of iced water, ammonia solution is added drop by drop until present in slight excess. The liquid should be alkaline to phenolphthalein after standing some hours; if not alkaline, more ammonia is added. After addition of sodium carbonate to render the hexamethylenetetramine more stable, the liquid is distilled until 100 c.c. has been collected. This distillate is neutralised with dilute sulphuric acid and fractionated through a "pear" still-head and the fraction passing over between 65° and 100° collected. This is again fractionated so as to obtain a distillate containing approximately 75% of methyl alcohol. In the final distillate the methyl alcohol is estimated by converting it into methyl iodide as already described. The second fractionation might be avoided and the process greatly simplified by diluting the distillate from sulphuric acid to some exact volume and determining the methyl alcohol present from the specific gravity.

A recent method, based on a different principle, is that of Blank and Finkenbeiner (*Ber.*, 1906, **39**, 1327). 1 grm. of the formalin is mixed with 50 c.c. of twice normal chromic acid (66.7 grm. chromic

acid (H_2CrO_4) per 1000 c.c.) and 20 c.c. of pure concentrated sulphuric acid (98%), and allowed to stand 12 hours, after which the mixture is diluted to 1000 c.c. To 50 c.c. of the diluted mixture a small crystal of potassium iodide is added, and the solution titrated back with N/10 thiosulphate.

EXAMPLE.—50 c.c. of the diluted solution, to which potassium iodide had been added, required not 50 c.c., but only 15 c.c. of N/10 thiosulphate. That is to say, oxygen equivalent to 35 c.c. of the thiosulphate has been used up in oxidising the formaldehyde and methyl alcohol contained in 0.05 grm. of the sample, or ($35 \times 1.6 =$) 56 grm. oxygen per 100 grm. of the sample. The latter was known to contain 40% of formaldehyde, 40 grm. of which require $\dfrac{40 \times 32}{30} = 42.7$ grm. oxygen. As methyl alcohol requires for its oxidation one and a half times its weight of oxygen, the percentage of methyl alcohol in the sample is $\frac{2}{3}$ ($56.0 - 42.7$) = 8.9%. The whole process can be completed in two hours if the oxidation be assisted by warming after the first violent action is over, but great care is necessary and the solution must not be evaporated below two-thirds its initial bulk.

For the **estimation of methyl alcohol in presence of ethyl alcohol,** the method of Thorpe and Holmes (*Trans. Chem. Soc.*, 1904, **85,** 1) is accurate, occupies but little of the analyst's time, and can be conducted in any laboratory. One other method will be described, as it is more rapid and in certain concentrations quite as accurate, but it depends on the use of an instrument which is not to be found in every laboratory. The method of Thorpe and Holmes depends on the complete oxidation of methyl alcohol to carbon dioxide by means of chromic acid mixture. Ethyl alcohol under the conditions of the experiment yields carbon dioxide equivalent to 0.5 % of its weight. The process is as follows:

"The sample is mixed with water in such proportions that 50 c.c. of the mixture shall contain not more than 1 grm. of methyl alcohol, and in the presence of ethyl alcohol not more than 4 grm. of the mixed alcohols. 50 c.c. of this mixture are then introduced into a 300 c.c. flask, which can be closed by a ground-in stopper and which is fitted with a funnel and side tube. 20 grm. of potassium dichromate and 80 c.c. of dilute sulphuric acid (1:4) are added, and the mixture allowed to remain 18 hours. A further quantity of 10 grm.

of potassium dichromate and 50 c.c. of sulphuric acid mixed with an equal volume of water are now added, and the contents of the flask heated to the boiling-point for about 10 minutes, the evolved carbon dioxide being swept out of the apparatus by a current of air and collected in soda-lime.

"When ethyl alcohol is present, a subtractive correction must be applied to the weight of carbon dioxide thus obtained in the proportion of o.o1 grm. of carbon dioxide for each grm. of ethyl alcohol present."

As in most cases the liquid to be examined will contain at least 10 times as much ethyl alcohol as methyl alcohol, the total alcoholic content may be determined with sufficient accuracy from the sp. gr. by reference to the ethyl alcohol tables.

In the above-described oxidation process acetone and methyl acetate are converted into acetic acid and carbon dioxide, while allyl alcohol is wholly oxidised yielding carbon dioxide. As the proportion of these substances in wood naphtha used for methylating in Great Britain is fairly constant, and as none of them are normal constituents of commercial ethyl alcohol, the fact that they take part in the reaction is of less importance where the object is to detect methylated spirit in tinctures or to estimate the proportion of wood naphtha in a sample of methylated spirit. The bulk of the secondary constituents of wood naphtha may be removed from the spirit by shaking with light petroleum and saturated salt solution; the alcohols are then recovered from the saline layer by distillation and submitted to the oxidation process. Even with this treatment the methyl alcohol will commonly be overestimated by 4% that is to say, 5.2% will be found where only 5% is present.

For the **estimation of methylated spirit in tinctures,** Thorpe and Holmes recommend that the spirit from 25 c.c. of the sample, or from 50 c.c. if it contains less than 50 per cent. of alcohol, be treated with light petroleum to remove essential oils, etc., as described in a later subsection (Estimation of Alcohol in Essences) and then distilled and diluted with water to a volume of 250 c.c.; 50 c.c. of this mixture is then oxidised with chromic acid mixture as above described. If the weight of carbon dioxide thus obtained does not exceed o.o1 grm. for each gram of alcohol present, this amount being equivalent to o.7 volume of methyl alcohol in 100 volumes of the alcohol, then it may be concluded that the sample contains only spirits of wine. Should the amount of

carbon dioxide exceed this amount, its equivalent in methyl alcohol by volume must be subjected to a subtractive correction of from 0.7 to 1% (depending on the amount of methylated spirit present), the the percentage of methylated spirit being calculated on the assumption that the quantity of methyl alcohol occurring in dehydrated methylated spirit does not exceed 8.8%.

An entirely different method's that of Leach and Lythgoe (*J. Amer. Chem. Soc.*, 1905, **27**, 964). It is based on the use of the Zeiss immersion refractometer, and when this instrument is available it affords the most rapid means of estimating methyl alcohol in presence of ethyl alcohol. With the immersion refractometer at 20°, distilled water gives a reading of 14.5 scale divisions. Addition of ethyl alcohol increases the reading; until at about 75% alcohol a maximum of 101 divisions is reached further addition of alcohol reduces the reading until at 100% alcohol it has fallen to 91. Small additions of methyl alcohol to water also increase the readings of the instrument, but to a lesser degree, and a maximum is reached at 50% alcohol when the reading is 39.8; further addition of methyl alcohol reduces the reading, so that at 91% it is again 14.9 or about the same as pure water, while at 100% alcohol the reading is only 2 divisions. Leach and Lythgoe give two tables, of which one is here reproduced, and an example of its use follows. The table shows the reading of the immersion refractometer corresponding to each percentage of alcohol, both ethyl and methyl, by weight, all readings being taken at exactly 20°. This table will show at a glance whether a solution of given strength of alcohol, as determined from the sp. gr., contains ethyl or methyl alcohol or is a mixture of the two.

For the estimation of methylated spirit in tinctures and essences it is necessary to obtain the alcohols-free from non-volatile matters and from essential oils before subjecting them to refractometric treatment. Leach and Lythgoe dilute 50 c.c. to 200 c.c., treat with magnesia, filter, distil 100 c.c. of the filtrate and make the volume of the distillate up to 100 c.c. The method of Thorpe and Holmes (see Estimation of Alcohol in Essences and Tinctures), in which the tincture is mixed with salt solution and the oils, etc., extracted with light petroleum, is preferable, as this treatment removes most of the acetone as well. By either of these methods the estimation of methyl alcohol is combined with that of ethyl alcohol and only requires the refractometric reading to be made on the distillate, the sp. gr.

SCALE READINGS ON ZEISS IMMERSION REFRACTOMETER AT 20°.
Corresponding to each % by weight of Ethyl and Methyl Alcohol.

Per Cent. alcohol by Weight.	Scale Readings.		Per Cent. Alcohol by Weight.	Scale Readings.	
	Methyl Alcohol.	Ethyl Alcohol.		Methyl Alcohol.	Ethyl Alcohol.
0	14.5	14.5	50	39.8	90.3
1	.8	16.0	51	.7	91.1
2	15.4	17.6	52	.6	.8
3	16.0	19.1	53	.6	92.4
4	.6	20.7	54	.5	93.0
5	17.2	22.3	55	.4	.6
6	.8	24.1	56	.2	94.1
7	18.4	25.9	57	.0	.7
8	19.0	27.8	58	38.6	95.2
9	.6	29.6	59	.3	.7
10	20.2	31.4	60	37.9	96.2
11	.8	33.2	61	.5	.7
12	21.4	35.0	62	.0	97.1
13	22.0	36.9	63	36.5	.5
14	.6	38.7	64	.0	98.0
15	23.2	40.5	65	35.5	.3
16	.9	42.5	66	.0	.7
17	24.5	44.5	67	34.5	99.1
18	25.2	46.5	68	.0	.4
19	.8	48.5	69	33.5	.7
20	26.5	50.5	70	.0	100.0
21	27.1	52.4	71	32.3	.2
22	.8	54.3	72	31.7	.4
23	28.4	56.3	73	.1	.6
24	29.1	58.2	74	30.4	.8
25	29.7	60.1	75	29.7	101.0
26	30.3	61.9	76	.0	.0
27	.9	63.7	77	28.3	100.9
28	31.6	65.5	78	27.6	.9
29	32.2	67.2	79	26.8	.8
30	.8	69.0	80	.0	.7
31	33.5	70.4	81	25.1	.6
32	34.1	71.7	82	24.3	.5
33	.7	73.1	83	23.6	.4
34	35.2	74.4	84	22.8	.3
35	.8	75.8	85	21.8	.1
36	36.3	76.9	86	20.8	99.8
37	.8	78.0	87	19.7	.5
38	37.3	79.1	88	18.6	.2
39	.7	80.2	89	17.3	98.9
40	38.1	81.3	90	16.1	.6
41	.4	82.3	91	14.9	.3
42	.8	83.3	92	13.7	97.8
43	39.2	84.2	93	12.4	.2
44	.3	85.2	94	11.0	96.4
45	.4	86.2	95	9.6	95.7
46	.5	87.0	96	8.2	94.9
47	.6	.8	97	6.7	.0
48	.7	88.7	98	5.1	93.0
49	.8	89.5	99	3.5	92.0
			100	2.0	91.0

of which has been taken to ascertain the total alcoholic strength. But where it is required to estimate small amounts of methylated spirit by the refractometer, it is well to redistil the distillate obtained by either of these methods and collect only the first 50 c.c. or even 25 c.c. The sp. gr. may show that a little alcohol has been lost, but a liquid is obtained of greater alcoholic strength which enables a part of the table to be used in which the readings of methyl and ethyl alcohols differ more widely.

EXAMPLE.—An orange extract was diluted four times with water, treated with magnesia and filtered. A measured portion of the filtrate was then distilled and the distillate made up to the measured portion taken. This distillate was found to have a sp. gr. of 0.9754 corresponding to 16.91% by weight, and to have a refraction of 42.0 on the Zeiss immersion refractometer. By interpolation in the table, the readings of ethyl and methyl alcohol corresponding to 16.91% alcohol are 44.3 and 24.45, respectively the difference being 19.85. 44.3 − 42.0 = 2.3. 100(2.3 + 19.85) = 11.6. Thus 11.6% of the alcohol present was methyl alcohol.

The following method, due to Riche and Bardy (*Compt. rend.*, 1875, **80**, 1076), though long and slow, is still valued by many experienced chemists. It depends on the formation of methyl aniline violet. 10 c.c. of the sample of alcohol, previously rectified if necessary over potassium carbonate, placed in a small flask with 15 grm. of iodine and 2 grm. of red phosphorus. Methyl and ethyl iodides are formed and should be distilled off into about 30 c.c of water. The heavy oily liquid which settles in the receiver is separated and transferred to a flask containing 5 c.c. of aniline. The flask should be placed in cold water, if the action is violent; or, if necessary, the reaction may be stimulated by gently warming the flask. After one hour the product is boiled with water and solution of rodium hydroxide added, when the bases rise as an oily layer, which may be drawn off with a pipette after filling the flask with water up to the neck. 1 c.c. of this oily liquid is oxidised by adding it to 10 grm. of a mixture of 100 parts of clean sand, 2 of common salt, and 3 of copper nitrate. After being thoroughly mixed, the mass is introduced into a glass tube and heated to 90° for eight or ten hours. The product is exhausted with warm alcohol, the liquid filtered, and made up with alcohol to 100 c.c. If the sample of spirit was pure, the tint of the liquid is red, but in presence of 1% of methyl alcohol it has a distinct

violet shade; with 2.5% the shade is very distinct, and still more so with 5%. To detect more minute quantities of methyl alcohol, dilute 5 c.c. of the colored liquid to 100 c.c. with water, and 5 c.c. of this again to 400 c.c. The liquid thus obtained is heated in porcelain, and a piece of undyed wool (8 cm. square is a convenient size) is immersed. The fabric should be cleaned before use with warm soap suds, washed thoroughly, and dried. It is stated that for this test the wool should be free from sulphur. The wool should be left in the liquid to be tested for about thirty minutes, then washed and dried. Pure alcohol will not produce a dye, but methylated alcohol will produce a violet, the depth of tint giving approximate indication of the proportion present. Comparison slips, made with 1, 2, 3 and 5% of methyl alcohol should be prepared as standards.

WOOD NAPHTHA. WOOD SPIRIT.

In addition to methyl alcohol and water, commercial wood naphtha contains acetone and higher ketones (nil to 14%), esters, mainly methyl acetate (nil to 4%), together with smaller proportions of allyl alcohol, pyridine and other substances.

For use in the colour industry a very pure spirit is required, acetone being a highly objectionable impurity, and as a consequence an acetone-free grade of spirit is now a regular article of commerce. For dissolving resins to make varnishes, the presence of acetone is an advantage on account of its solvent properties. As a denaturant of ethyl alcohol, the proportion of nauseous constituents—allyl alcohol, pyridine, etc.—is most important.

ASSAY OF WOOD NAPHTHA.

The methods now to be described are applicable not only to crude or partially purified naphtha, but to any commercial sample of wood spirit. The impurities to be looked for and estimated are the same in every grade of spirit up to the so-called acetone-free grade; the various grades differ only in the amount of the secondary constituents, with the reserva ion that some crude, usually high-coloured, naphthas contain impurities of high boiling-point (150–200° and over), hydrocarbons, acids and bases, which are scarcely detectable in spirit of average quality.

Estimation of Methyl Alcohol.—The British Government Laboratory method has been given in an earlier subsection (p. 91).

Estimation of Acetone and Higher Ketones.—These are best estimated by Messenger's method (*Ber.*, 1888, 21, 3366) and calculated as acetone. The method is rapid and with solutions of pure acetone in water or methyl alcohol gives excellent results. Applied to wood naphtha it is less satisfactory; the whole of the acetone reacts no doubt, so that the number obtained is not less than the amount of real acetone present. Certain higher ketones, which are present, also take part in the reaction, but reduce a smaller amount of iodine per unit of weight than does acetone, so that the number obtained is lower than the true amount of ketones present. The method is, however, as good as any and the quickest yet described. That of Denigès (*Bull. Soc. chim.*, 1899 (v), 19, 754), based upon the formation of an insoluble compound of acetone with mercuric sulphate, is open to similar objection and it occupies more time. Messenger's method is followed with but slight modification in the Br tish Government Laboratory, where the following procedure is adopted:

"25 c.c. of N/1 sodium hydroxide are placed in a stoppered flask of about 200 c.c. capacity. To this is added 0.5 c.c. of the naphtha. The mixture is well shaken and allowed to stand 5 to 10 minutes. Into it from a burette N/5 iodine solution is run slowly, drop by drop, vigorously shaking all the time, till the upper portion of the solution, on standing a minute, becomes quite clear. A few c.c. more of N/5 iodine solution are added, as to get concordant results an excess of at least 25% of the iodine required must be added. After shaking, the mixture is allowed to stand for 10 to 15 minutes, and then 25 c.c. N/1 sulphuric acid are added. The excess of iodine is liberated, titrated with N/10 sodium thiosulphate solution and starch, and half the number of c.c. of thiosulphate solution used are deducted from the total number of c.c. of iodine so ution used. The difference gives the amount of acetone by weight in the naphtha by the formula: c.c. N/5 iodine solution required × 0.387 = grm. of acetone per 100 c.c. of wood naphtha.

"This includes as acetone any aldehydes, etc., capable of yielding iodoform by this reaction.

"If the quantity of 'acetone' is excessive, a less quantity of the spirit is taken, or 10 c.c. are diluted with 10 c.c. of methyl alcohol free from acetone, and 0.5 c.c. of the mixture is used."

Messenger in his original communication calls attention to the fact that commercial sodium hydroxide may contain nitrite, which must be allowed for. This is easily done by adding a crystal of potassium iodide to 25 c.c. of the N/1 sodium hydroxide, acidifying and titrating against the standard thiosulphate. It is, however, not difficult to obtain sodium hydroxide which is practically free from every impurity except water.

Estimation of Esters.—To 5 c.c. of the naphtha contained in a small Jena glass flask, 20 c.c. of recently boiled distilled water are added, and then 10 c.c. of N/1 sodium hydroxide and the whole heated for two hours under a reflux condenser on the water-bath. The liquid is then cooled, phenolphthalein added and the excess of sodium hydroxide titrated with N/1 acid.

Let the amount of acid required be x c.c. Then the number of grm. of esters (calculated as methyl acetate) in 100 c.c. of the sample is 1.48 (10 — x). If the proportion of esters is very small and it is required to estimate them with great accuracy, a much larger quantity of spirit may be taken, and N/10 alkali and acid used, but in this case the spirit should be first boiled under a reflux condenser to expel carbon dioxide. Wood spirit is generally almost neutral to phenolphthalein, but if not it must of course be rendered neutral before proceeding to the estimation of esters. In the British Government Laboratory the hydrolysis of the esters is conveniently effected in a silver pressure flask of about 150 c.c. capacity.

Bromine Test for Unsaturated Compounds.—No accurate method for the estimation of allyl alcohol in wood naphtha exists, but the amount of bromine which the naphtha will decolourise is some measure of the unsaturated compounds, of which allyl alcohol is known to be one commonly present. For denaturing spirits of wine in Britain, wood naphtha is required to have a certain minimum capacity for decolourising bromine; not more than 30 c.c. of the naphtha must be necessary to decolourise 0.5 grm. bromine. The test is conducted as follows:

A standard bromine solution is made by dissolving 12.406 grm. of potassium bromide and 3.481 grm. of potassium bromate in a litre of recently boiled distilled water.

50 c.c. of this standard solution (= 0.5 grm. bromine) are placed in a flask of about 200 c.c. capacity, having a well-ground stopper. To this is added 10 c.c. of dilute sulphuric acid (1 in 4) and the whole

shaken gently. After standing for a few minutes the wood naphtha is slowly run from a burette into the clear brown solution of bromine until the latter is completely decolourised.

Estimation of Basic Substances.—These (pyridines, mono-, di-, and trimethylamine,) can be to some extent measured by the methyl-orange alkalinity of the sample, though as it is not known which base predominates it is not usual to calculate the bases, as pyridine for instance, in the manner in which the ketones are calculated as acetone and the esters as methyl acetate. Fawsitt, to whom we owe much of our information concerning wood alcohol, mentions the methylamines, but not pyridine, which is certainly present in most samples. But no doubt the bases in naphthas differ with the source of the naphtha. For use as a denaturant in Britain, wood naphtha must comply with the following specification as to reaction with indicators:

"The naphtha should be faintly acid to phenolphthalein, slightly alkaline or neutral, rarely acid to litmus, and always alkaline to methyl orange. 25 c.c. of the wood naphtha are placed in each of two beakers, and titrated with N/10 acid, using in one case a few drops of litmus solution, and in the other of a solution of methyl orange as indicator. With litmus usually 0.1 to 0.2 c.c. of N/10 acid is required to neutralise. With methyl orange the total alkalinity should be greater, at least 5 or 6 c.c. of N/10 acid being required for neutralisation.

" The total alkalinity, less that given with litmus, is the 'methyl orange alkalinity' and, for the 25 c.c. of wood spirit, should not be less than is required to neutralise 5 c.c. of N/10 acid."

Furfural may be detected by adding 10 c.c. of the naphtha to 1 or 2 c.c. of acetic acid in which a few drops of aniline have been dissolved. Furfural if present will develop an intense red, but as acetic acid contains furfural quite as often as wood spirit does, the acid and aniline must be mixed first and must remain colourless for five minutes before the spirit is added.

Wood spirit occasionally contains 1% or more of substances of high boiling-point (150° to 200° and over) which may be separated simply by slow distillation of a large quantity on the water-bath. As the residue is tar, the method to be adopted for its further examination must be looked for in another section. This tar has the characteristic offensive odor of crude naphtha, only in a greater degree. Sepa-

rated into hydrocarbons, phenolic bodies and bases, only the latter are found to be offensive; the hydrocarbons have the odor of terpenes while the predominant phenolic body is no doubt guaiacol, which alone has a grateful odour, but in combination with pyridine bases it appears to make their offensive odour even more offensive.

The detection of small admixtures of *ethyl alcohol* in wood spirit is less important than the converse. The following tests have been proposed for the purpose:

Berthelot suggested heating the sample with twice its volume of concentrated sulphuric acid. If 1% of ethyl alcohol is present, ethylene is evolved, and may be absorbed by bromine and estimated as ethylene dibromide. Acetone and the normal impurities of wood spirit may yield carbon monoxide and carbon dioxide but not ethylene.

Riche and Bardy (*Compt. rend.*, 1876, **82**, 768) use a reaction dependent on the production of aldehyde from ethyl alcohol by oxidising agents, and the action of aldehyde, methylal, acetal, etc., on salts of rosaniline, whereby a violet coloring matter is produced, which is not destroyed by subsequent addition of sulphurous acid. 4 c.c. of the liquid to be examined are mixed with 6 c.c. of concentrated sulphuric acid and 10 c.c. of water. 7 or 8 c.c. are distilled into 10 c.c. of water, and to this liquid are added 5 c.c. of sulphuric acid and 10 c.c. of a solution of potassium permanganate of 1.028 sp. gr. After five minutes have elapsed, 4 c.c. of a solution of sodium thiosulphate, of 1.29 sp. gr., and 4 c.c. of a solution of magenta, containing 0.02 gram per 1000 c.c. are added. Under hese conditions, wood spirit unmixed with ethyl alcohol gives a yellowish-white liquid but if ethyl alcohol is present the solution assumes a violet color of greater or less intensity. Acetone, formic acid, and isopropyl alcohol give no similar reaction.

For the examination of a reputed high-grade methyl alcohol, the above tests will require little variation, except in the case of the esters when the procedure is that described when dealing with these. Pure methyl alcohol should of course be neutral to both phenolphthalein and methyl orange, it should give no iodoform reaction and it should not decolourise bromine. If a spirit passes all these tests, its content of methyl alcohol may safely be deduced from its sp. gr.

The specifications with which wood naphtha, intended for methylating, has to comply differ considerably in Great Britain and the United States.

In Great Britain the wood naphtha must be sufficiently impure to impart to the methylated spirit such an amount of nauseousness as will, in the opinion of the Principal of the Government Laboratory, render such mixture incapable of being used as a beverage or of being mixed with potable spirits of any kind without rendering them unfit for human consumption. It must conform to the following tests:

a. Not more than 30 c.c. should be required to decolourise a solution containing 0.5 grm. of bromine.

b. It should be neutral or only slightly alkaline to litmus, and 25 c.c. should require at least 5 c.c. of N/10 acid when methyl orange is used as indicator.

It should contain:

a. At least 72 % by volume of methyl alcohol.

b. Not more than 12 grm. per 100 c.c. of acetone, aldehydes and higher ketones, estimated as "Acetone" by Messenger's method.

c. Not more than 3 grm. per 100 c.c. of esters, estimated as methyl acetate by hydrolysis.

In the United States the colour must not exceed that of N/5000 iodine solution, the sp. gr. must not exceed 0.830 at 60°/60° F., and 90% should distil below 75° at 760 mm. Diluted with a double volume of water, the naphtha should remain clear or develop only a slight opalescence. The "acetone" by Messenger's method must not be less than 15 nor more than 25 grm. per 100 c.c., and the esters must not exceed 5 grm. per 100 c.c. Not less than 15, nor more than 25 c.c. should be required to decolourise a solution containing 0.5 grm. of bromine.

ACETONE.

Dimethyl-ketone, $CH_3.CO.CH_3$.

For some reason, presumably because it is an important constituent of wood spirit, acetone has found its way into this section of this work, and though it is not an alcohol, this arrangement is continued rather than make further departure from the original plan.

Acetone is a colourless, pleasant-smelling, neutral liquid, miscible in all proportions with water, methyl alcohol and ethyl alcohol. It is said to be thrown out of its aqueous or alcoholic solution by saturating these with calcium chloride. From its aqueous or dilute alcoholic solution this is more or less true, and if petroleum spirit is added the separation is fairly complete and common salt may replace the calcium chloride.

From strong methyl alcohol the separation by means of calcium chloride is much more difficult than might be expected from the published statements. The b. p, according to Fuchs (*Zeitsch. angew. Chem.*, 1898, **38**, 870), ranges from 55.06 at 710° mm. to 58.16° at 790 mm. The m. p. according to Ladenburg and Krugel (*Ber.*, 1899, **32**, 1821), is —94.9°. The sp. gr. at 15°/4°, according to MacElroy and Krug (*J. Anal. Chem.*, **6**, 187), is 0.79726. These authors give a table showing sp. gr. at 15°/4° of aqueous solutions of acetone and the table has been reprinted in the *Chem. Centr.*, 1892, **2**, 158, and in the *J. Chem. Soc.*, 1893, **64**, i, 7.

Detection of Acetone.—For the detection of acetone, especially in urine, a great many tests have been described. Only three will be referred to here.

Lieben's Iodoform Test.—Acetone gives the iodoform reaction in the cold and, in the probable absence of other substances, such as aldehyde and isopropyl alcohol which behave similarly, the formation of iodoform in the cold is useful as a test for acetone. To 2 c.c. of the liquid, 3 to 5 drops of 10% sodium hydroxide are added and then, drop by drop, N/2 iodine solution until very faintly yellow. In the presence of acetone, iodoform separates at once.

Legal's Nitroprusside Test.—To 5 c.c. of the liquid 5 drops of a then 1 c.c. of 10 % sodium hydroxide solution. In presence of freshly-prepared solution of sodium nitroprusside are added, and acetone the liquid assumes an orange tint, which fades to clear yellow in 15 to 20 minutes. If the experiment be repeated and the solution made just distinctly acid with acetic acid immediately after the addition of the alkali, a purplish-red color will develop in presence of acetone, and this colour remains practically unchanged for 15 to 20 minutes. The comparative persistence of this purple color (it slowly changes, becoming more blue) serves to distinguish acetone from higher homologues and from certain other substances which may occur in urine.

Salicylaldehyde Test.—This test (Frommer, *Berl. klin. Wochensch.*, 1905, **42**, 1008) is said to be the most delicate yet described. It is given third place here, because salicylaldehyde, unlike iodine and nitroprusside, is not always at the analyst's command. To 10 c.c. of the liquid to be examined 1 grm. of solid potassium hydroxide is added and then, without waiting for this to dissolve, 10 drops of salicylaldehyde, and the whole warmed to 70°. In the presence of acetone a purple-red contact-ring develops. If the hydroxide is all

dissolved before the addition of the salicylaldehyde, the liquid becomes yellow, then reddish, and finally purple-red.

Detection of Acetone in Urine.—R. L. Siau, who with F. W. Pavy has done perhaps more than any other worker in this field, informs the writer that the detection and estimation of acetone in urine has not the importance which the voluminous literature might suggest, but since the analyst is frequently asked to carry out such tests, the subject must be dealt with, even if very briefly. For an exhaustive review of the many methods which have been described, those specially interested are referred to a series of papers by Bohrisch (*Pharmaceut. Centralho*, 1907, **48,** 181, 206, 220, and 245). Bohrisch recommends the analyst not to rely on any one test, but to apply two or three. The salicylaldehyde test may be applied to the urine direct and it has the advantage that aceto-acetic acid, which may be present in pathologic urine, does not give the reaction. If no red or reddish ring develops, but only a yellow colouration, acetone is certainly absent. On the other hand, the reaction is so sensitive that it gives no idea of the quantity present. If a positive result is obtained, therefore, a less delicate test should be applied, preferably the nitroprusside test, which may also be applied to the urine direct without distillation or other previous treatment. A positive result indicates a notable quantity of either acetone or aceto-acetic acid. To distinguish between these, many methods have been described. Bohrisch recommends acidifying 50 c.c. of the urine with sulphuric acid and shaking with 25 c.c. of ether. The ether is then shaken with 15 to 20 c.c. of water, which will then contain a large part of the acetone and aceto-acetic acid originally present, while other substances which interfere with the tests subsequently to be applied are got rid of. The aqueous layer is freed from dissolved ether by warming to 40° with frequent shaking, and a portion is then tested for aceto-acetic acid by means of ferric chloride. If no violet colouration results, then the positive reaction with nitroprusside must have been due to acetone. But if aceto-acetic acid is shown to be present, the remainder of the ether-free aqueous extract is tested for acetone by the iodoform test. This test should not be applied to urine direct, because other substances which give the reaction may be present, and the above-described method of shaking out, though far from quantitative, is preferable to any distillation method, because at the temperature of distillation aceto-acetic acid and other substances may be decomposed, yielding acetone.

Estimation of Acetone.—Messenger's volumetric method (described under Assay of Wood Spirit) maintains its position as that most frequently applied. In common with every other method, it gives less satisfactory results with complex mixtures than with pure aqueous or alcoholic solutions of acetone, since the results are influenced by the presence of anything which can reduce iodine in the cold. It is more accurate as well as more expeditious than the gravimetric method of Krämer (*Ber.*, 1880, **13,** 1000) from which it was developed, though a return to this is periodically recommended. Its critics, as a rule, only recommend the substitution of some other reducing solution for the thiosulphate, whereas what is needed is not a different solution, but a sufficient excess of iodine and time for the reaction to take place. The British Government Laboratory directions for carrying out the determination are reproduced in this edition, because in those directions proper account has been taken of hese factors.

Method of Jolles.—This author (*Ber.*, 1906, **39,** 1306) has found that the reaction, $CH_3.CO.CH_3 + NaHSO_3 = CH_3.C(OH)(SO_3Na).CH_3,$ proceeds quantitatively with respect to the acetone, if the sulphite is present in large excess and sufficient time allowed, and on this observation has based the following method for the estimation of acetone:

A solution of sodium hydrogen sulphite is prepared of known titre with respect to iodine, and a large excess (three or four times as much as is likely to be required) added to a measured or weighed portion of the liquid to be tested. After 30 hours the excess of sulphite is titrated with standard iodine solution. One mol. of sodium hydrogen sulphite or two atoms of iodine correspond to one mol. of acetone.

Method of Denigès.—(*Compt. rend.*, 1898, **127,** 963, and *Bull. Soc. chim.*, 1899, [v], **19,**754.) This method depends on the quantitative formation of an insoluble compound of definite composition when acetone is treated with a large excess of mercuric sulphate. The reagent is prepared by dissolving 5 grm. of mercuric oxide in 100 c.c. of water to which 20 c.c. of sulphuric acid has been added. If it is desired to make use of the formula given by Denigès for simplifying the calculation in the volumetric modification of the method, it is necessary to make up this solution so that each 100 c.c. contains exactly 5 grm. of mercuric oxide or preferably to carry out a blank experiment. The liquid under examination is, if necessary, diluted with water until its content of acetone is reduced to 0.2%; the

content of methyl alcohol also must not exceed 50% nor that of ethyl alcohol 2 %. To 25 c.c. of the diluted liquid 25 c.c. of of the mercury reagent is added, and the whole heated on the water-bath for ten minutes. After cooling, the precipitate is collected on a tared filter, washed with not more than 100 c.c. of cold water, dried at 100°. and weighed as $3Hg_sS_2O_{11}.4C_3H_6O$). The weight of the precipitate multiplied by 0.0609 gives the weight of acetone in the 25 c.c. of liquid taken for the experiment.

Volumetric modification of Denigès' method. This depends on the use of a mercury solution of exactly known strength, and the estimation of the mercury remaining in the filtrate from the precipitate of the acetone compound. The procedure is the same as in the gravimetric method up to the filtration, except that the volume of reagent taken must be exactly measured. The washing of the precipitate is stopped when the filtrate and washings amount to nearly 100 c.c., the volume made up exactly to 100 c.c. and the contents of the flask shaken. To 20 c.c. of this liquid mixed with 15 c.c. of ammonia and not less than 50 c.c. of water, 10 c.c. of potassium cyanide solution (13 grm. cyanide per litre) are added. This is rather more than equivalent to the mercury which can be present even if none has been removed by acetone. If much mercury has been removed from solu- and this excess is now titrated with N/10 silver nitrate, using potas- tion by acetone, there will be a large excess of potassium cyanide, sium iodide as indicator until there is a slight permanent turbidity. If n be the number of c.c. of silver solution required and x the percentage of acetone in the liquid, of which 25 c.c. were taken for the test, then

$$x = (n-0.4) \times 0.31.$$

In this formula 0.4 is the volume of silver solution which would be required if there were no acetone present and if the solutions of potassium cyanide and mercuric sulphate were of the exact strength stated. As these solutions will rarely be exact in titre, it is better to carry through a blank experiment with the measured quantities stated, but no acetone, and to substitute the volume of silver nitrate required under these conditions for the 0.4 of Denigès' formula.

Estimation of Acetone in Urine.—Distillation and estimation of the acetone in the distillate by Messenger's method is most usual. Aceto-acetic acid if present will be decomposed at the tem-

perature of distillation, yielding acetone. For their separate estimation Folin has described a method (*J. Biol. Chem.*, 1907, **3**, 177), but the subject is not sufficiently important to justify a description detailed enough to obviate reference to the original.

Assay of Commercial Acetone.—The British War Office specification requires acetone for cordite manufacture to be colourless and absolutely transparent, and when mixed with distilled water in any ⌐ when evaporated on a boiling water-bath, and its sp. gr. at 15°/15° ⸜proportion it must show no turbidity. It must leave no residue must not exceed 0.800. It must also endure the "permanganate test" for 30 minutes. The permanganate test is conducted as follows: 100 c.c. of the acetone is mixed with 1 c.c. of 0.1 % potassium permanganate solution, kept at a temperature of 15° and the time observed for the colour of the permanganate to disappear. This specification is said to ensure the absence of any impurity other than a very small quantity of ethyl methyl ketone. Since 1904 the folowing clause has been added to the specification:

"The acetone is not to contain more than 0.002 % of carbon dioxide, and is otherwise to be quite neutral."

In titrating acetone with acid or alkali it is well to dilute it with an equal bulk of recently-boiled distilled water. For the estimation of basic bodies and strong acids *p*-nitrophenol is usually employed as indicator, while for weak acids phenolphthalein is used after boiling to expel carbon dioxide. The latter is estimated by titration of the unboiled sample, using phenolphthalein as indicator. (Marshall, *J. Soc. Chem. Ind.*, 1904, **23**, 646.)

In examining samples of acetone, less carefully fractionated, the following observations of Heikel (*Chem. Zeit.*, 1908, **32**, 75) are useful. The higher ketones react with iodine and with mercuric sulphate, but consume less of the reagent per unit of weight than does acetone. Thus the fraction known in the trade as "ketones" (mainly ethyl methyl ketone), sp. gr. 0.811 to 0.815, appears to contain 90% of acetone by Messenger's method and 63.5% by that of Denigès. The "light acetone oil" of the trade, sp. gr. 0.82 to 0.83, appears to contain 57% of acetone by Messenger's method and only 32.5% by that of Denigès. This fraction contains little or no acetone really, but it is the apparent acetone content by the two methods or the Denigès-Messenger ratio which is valuable in judging the sample. For acetone itself the ratio is of course 1, for "ketones" as the above numbers

show about 0.7, and for light oil about 0.57. Moreover, the mercury precipitate with "ketones" is no longer white, but yellowish, while that from the light oils is yellowish-brown.

ETHYL ALCOHOL.

Alcohol, Methyl Carbinol.

Pure ethyl alcohol is a colourless, nearly odourless, mobile liquid, possessed of a burning taste. Its b. p. varies from $76.36°$ at 710 mm. to $79.31°$ at 790 mm. (Fuchs, *Zeitsch. angew. Chem.*, 1898, **38**, 870). It solidifies at $—112.3°$ (Ladenburg and Krugel, *Ber.*, 1899, **32**, 1818). The sp. gr. is probably not far from 0.79394 at $60°/60°$ F., the temperature adopted for alcoholometry in Britain. The numbers of Mendeléef—(*Zeitsch. Chem.*, 1865, 260, and *Ann. Phys. Chem.*, 1869, **138**, ii, 138, 103 and 250), of Young (*Trans. Chem. Soc.*, 1902, **81**, 717), and of Klason and Norlin (*Arkiv Kem. Min. Geol.*, 1906, **2**, No. 24), when calculated to $60°/60°$ F., become 0.79393, 0.79395 and 0.79394, respectively. Squibb (*Ephemeris*, 1884, **2**, 522, and *Pharm. J.* (3), **15**, 22) has obtained alcohol of sp. gr. 0.79350, and there is a natural presumption in favour of the lowest recorded number, but Young's method of preparation makes it unlikely that his number is higher than the truth and he suggests that Squibb's alcohol contained ether. The tables of specific gravities of aqueous alcohol most commonly in use are based on the earlier (1847–8) work of Fownes and Drinkwater, using alcohol of a sp. gr. of 0.7938, while the tables of Tralles, which alcohol has a sp. gr. of 0.7946, are still the basis of the excise work in Britain. The co-efficient of expansion is very large, a point of considerable importance to the analyst, who usually estimates alcohol from the sp. gr. of its aqueous solutions.

Alcohol is miscible with water in all proportions, a considerable evolution of heat and contraction in bulk taking place on admixture.

The presence of as small a proportion as 0.5% of water in alcohol is indicated by the pink color assumed by the liquid on introducing a crystal of potassium permanganate. A less delicate test consists in agitating the alcohol with a little anhydrous cupric sulphate, when the salt will acquire a blue colour if a notable quantity of water be present.

According to P. Yvon (*J. Pharm. Chim.*, 1897, **7**, 100), calcium carbide furnishes a ready means of determining whether alcohol is anhydrous or not. On adding a pinch of the powder to absolute alcohol, no bubbles of gas are liberated and the liquid remains transparent,

whilst if only a trace of water is present bubbles of acetylene are liberated and the liquid becomes milky from the formation of calcium hydroxide.

Rectified Spirit of Wine is the name given to the most concentrated alcohol producible by ordinary distillation. The rectified spirit of the British Pharmacopœa is described as containing 84% by weight of real alcohol, and having a sp. gr. of 0.838.

Proof Spirit of the British Pharmacopœia has a sp. gr. of 0.920, which corresponds to a strength of about 49% by weight of real alcohol. The term "proof spirit" is very confusing to many people, and might with advantage be abandoned. Of this there is little chance at present, as it is adopted in several Acts of Parliament, and is the scale to which Sykes' hydrometer, used by the Excise, has reference. The Excise formerly tested the strength of spirits by pouring a certain amount on gunpowder. A light was then applied. If the spirit was above a certain strength ("proof") the gunpowder ultimately inflamed, but if weaker the gunpowder was too much moistened by the water to be capable of explosion, and the sample was said to be "under proof." By Act of Parliament, proof spirit is now defined to be a liquid of such density that, at 51° F., 13 volumes shall weigh the same as 12 volumes of water at the same temperature. The "proof spirit" thus produced has a sp. gr. of 0.91984 at 60°/60° F., and contains, according to Fownes, 49.24% by weight of alcohol and 50.76 of water. Spirits *weaker* than the above are described by the Excise as being so many degrees, or so much % "under proof" (U.P.). Thus, by the term "spirit of 20% or 20 degrees, under proof," is meant a liquid containing, at 60° F., 80 volumes of proof spirit and 20 of water. "Spirit of 50° U.P." contains equal volumes of proof spirit and water, while pure water is 100° under proof.

On the other hand, spirituous liquids *stronger* than proof spirit are described according to the number of volumes of proof spirit 100 volumes would yield when suitably diluted with water. Thus, "spirit of 50° O.P." is alcohol of such strength that 100 volumes at 60° F., when diluted with water to 150 volumes, would be proof spirit.[1] Absolute alcohol accordingly is 75 ¼° O.P., and contains 175.25% of proof spirit, for 100 volumes when diluted with water would yield 175.25 volumes of spirit at "proof."

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[1] Owing to the contraction which occurs on mixing alcohol with water, the volume of water which it would be necessary to add in this instance would be considerably *more* than 50 measures. Thus, a mixture of 100 volumes of absolute alcohol with 60 of water only measures 154 volumes instead of 160.

The relationship of percentages of absolute alcohol to those of proof spirit are explained below.

In the United States, Tralles' tables are legalised, and consequently the proportion of alcohol in spirit is usually stated in percentage by volume; but a "proof spirit" is also recognized by the American Excise, which is defined as "that alcoholic liquor which contains one-half its volume of alcohol of a specific gravity of 0.7939 at 60°. The sp. gr. of such spirit is stated to be 0.93353 at 60° F., water at its maximum density being taken as unity. (This will correspond to a sp. gr. of about 0.9341 if water at 60° F. be taken as unity, and to a content of 42.7% by weight of absolute alcohol.) Absolute alcohol would contain 200% of proof spirit according to the United States Excise, instead of 175.25% in the English system.[1]

The United States Pharmacopœia designates three forms of alcohol:

Absolute Alcohol.—At least 99% by weight of ethyl hydroxide. Sp. gr. at 15.6° not above 0.797; at 25° 0.789.

Alcohol.—91% by weight or 94% by volume of ethyl hydroxide. Sp. gr. at 15.6° 0.820; at 25° 0.812.

Diluted Alcohol.—About 41% by weight or 48.6% by volume of ethyl hydroxide. Sp. gr. at 15.6° 0.937; at 25° 0.930.

Examination of Commercial Alcohol.—Ordinary spirit of wine is commonly assumed to consist of only alcohol and water. This, however, is frequently far from true, commercial alcohol often containing distinct traces of higher homologues, of aldehyde and acetic acid, of volatile oils and of various fixed impurities, both organic and inorganic. *Methylated spirit of wine* is an acknowledged mixture of ethyl alcohol and *wood spirit*. For the detection of the latter body in alcoholic liquids in which its unacknowledged presence is suspected, see Methyl Alcohol.

The other common impurities of commercial alcohol may be sought for by the methods given for the analysis of potable spirits.

Oily and Resinous Matters may be detected by diluting the spirit somewhat largely, when they are precipitated and impart a milky appearance to the liquid.

Aldehyde imparts a peculiar flavour to the spirit. When present in

[1] "*High Wines.*" This term is applied in the United States to the commercial alcohol o high strength, the amount of alcohol being usually indicated U. S. proof gallons. 95% alcohol is termed 190° proof.

"*Cologne Spirit*" "*Silent Spirit.*" These terms are much used to designate strong alcohol that has been freed from all but very small amounts of accessory substances. It has a very faint odour. Such alcohol is used in manufacture of toilet preparations, blended whiskies and imitations of many alcoholic beverages.—H. L.

quantity the spirit becomes brown when heated with sodium hydroxide. A smaller quantity is detected by adding a few drops of solution of silver nitrate and exposing the liquid to a good light for twenty-four hours, when the silver will be reduced and deposited as a black powder if aldehyde or other reducing agent be present. Traces of aldehyde are nearly always present in commercial samples of alcohol. The British Pharmacopœia directs the silver test to be made by adding 30 fluid grains (2 c.c.) of N/10 silver nitrate to 4 fluid ounces (120 c.c.) of the sample to be tested. After exposure to the light for twenty-four hours and decantation from the black precipitate, no further reduction of silver should occur on repeating the treatment. A negative result on adding more silver solution and again exposing the liquid to light proves the absence of a greater proportion of reducing agents per pint (250 c.c.) of spirit than can decompose about 2.5 grains (0.6 grm.) of nitrate of silver.

The proportion of *water* present in commercial alcohol may be deduced with accuracy from the sp. gr. of the liquid (p. 115).

Denatured Alcohol.—Spirit, suitably denatured so as to be unfit for drinking purposes, is free from duty in most countries. Formerly in Great Britain it was only necessary to add to the spirit one-ninth of its volume of partially purified wood naphtha. The British and United States specifications with which wood naphtha intended for methylating has to comply are given in the section on Methyl Alcohol. Since spirit denatured in this way can be deprived of its offensive taste and odour without great difficulty, the British Board of Inland Revenue subsequently directed the further addition of three-eighths of 1 % of mineral naphtha. In view of representations which were made to the Board that British manufacturers were placed at a disadvantage as compared with their foreign competitors by the regulations with regard to the use of duty-free alcohol for industrial purposes, these regulations have been revised, with the result that a very large number of denaturants are now permitted in place of crude wood naphtha. In these circumstances, a method for estimating crude benzene in alcohol is worth description.

For the *estimation of crude benzene in alcohol*, Holde and Winterfeld (*Chem. Zeit.*, 1908, 32, 313) take 100 c.c. of the spirit, dilute it with water so that the alcoholic strength is reduced to approximately 25% distil, collect the first 10 c.c. of the distillate in an ice-cooled pump-flask, dilute it with 10 to 20 c.c. of water and pour the mixture

into a narrow measuring cylinder. About 0.3 c.c. of benzenes remain emulsified in the 25 c.c. or so of dilute alcohol, but any larger quantity forms a separate upper layer, the volume of which is read off and 0.3 c.c. added to the reading. With quantities between 0.5 and 5.0% the maximum error should not exceed 0.1 per cent.

Methylated Finish is a preparation sold by those who are not licensed as venders of methylated spirit. It is made by dissolving a gum-resin in methylated spirit, and the British Excise insists that the proportion present shall not be less than 3 ounces in the gallon.

Detection of Alcohol.—The mere detection of alcohol is seldom important and, as a rule, it can be estimated quickly and with considerable accuracy. Methods for its detection in ether, chloroform and some other liquids from which it cannot be easily separated by distillation are described in the sections relating to those substances.

✓ Alcohol gives the iodoform reaction (see detection of acetone), but only on warming, preferably to about 60° for one minute. This reaction can often be applied to the detection of alcohol, although it is given by many other substances. Of these substances, some give the reaction in the cold, others only after prolonged warming. If an aqueous liquid, on being neutralised and once or twice fractionally distilled through some simple head, yields a distillate which has a sp. gr. notably less than 1, and which gives the iodoform reaction, but only on warming, the presence of alcohol may be suspected, and in many cases the presence of any of the other substances which might depress the sp. gr. and give the iodoform reaction is so improbable that such a distillate is practically a proof of the presence of alcohol. There is almost no limit to the sensitiveness of this test, if the number of distillations are increased.

E. Merck (*Chem. Zeit.*, 1896, **20**, 228) proposes the following modification of Davy's test: Pure molybdic acid is dissolved in warm strong sulphuric acid, and the resulting solution poured through the liquid under examination in a test-tube, both being kept as nearly as possible at a temperature of 60°. In presence of alcohol a blue ring appears at the junction between the two liquids, which is the more intense the larger the proportion of alcohol present. On shaking, the colour disappears, but by addition of a further quantity of the reagent it may be reproduced. The test is, of course, not characteristic of alcohol only, but it will detect even 0.02% of ethyl alcohol and 0.2% of methyl alcohol in aqueous solution.

Estimation of Alcohol.—Alcohol in admixture with wood spirit, chloroform, ether, etc., may be estimated by the methods described in the sections devoted to these substances. In by far the greater number of instances the estimation of alcohol is effected by *separating it from fixed substances* by distillation, and then ascertaining the proportion of alcohol present in the spirituous liquid condensed. This is practically the

Estimation of Alcohol in Mixtures consisting essentially of Alcohol and Water only.

This is most generally effected by ascertaining the sp. gr. of the mixture. From the sp. gr. at 60° F. compared with water at 60° F., the percentage of real alcohol is readily ascertained by reference to tables, on the construction of which great care has been bestowed by various observers, the subject being of great importance for excise purposes. By the excise, a glass or metal hydrometer is employed, the temperature of the liquid being carefully noted. In the laboratory, the specific-gravity bottle is a more satisfactory and accurate instrument. A bottle holding 50 c.c. is of suitable capacity for general use, but for some purposes a smaller one or a 10 c.c. Sprengel tube will be found serviceable. (See page 5.)

The proportion of alcohol contained in spirituous liquids is expressed in three ways: 1. Percentage of alcohol by weight. 2. Percentage of alcohol by volume. 3. Percentage of proof spirit. Of these, the first, in the opinion of the author, is the most satisfactory, but both the other plans serve for certain purposes. It is convenient in some cases to know the weight of alcohol in 100 measures of the spirituous liquid. The term "proof spirit" has already been explained.

In the following table are given the percentages of absolute alcohol by weight and of proof spirit by volume, which are contained in mixtures of alcohol and water of different sp. gr.:

SPECIFIC GRAVITIES OF MIXTURES OF ALCOHOL WITH WATER.

Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight	Percentage of Proof Spirit by Measure	Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight	Percentage of Proof Spirit by Measure
.79384	100.00	175.25	.798	98.66	173.81
.794	99.94	175.18	9	.34	.47
5	.61	174.83	.800	.03	.14
6	.29	.49	1	97.70	172.77
7	98.97	.14	2	.37	.39

SPECIFIC GRAVITIES OF MIXTURES OF ALCOHOL WITH WATER.—Continued.

Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.	Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.
.803	97.03	172.02	.850	79.32	148.84
4	96.70	171.64	1	78.92	.27
5	.37	.26	2	.52	147.69
6	.03	170.88	3	.12	.11
7	95.68	.46	4	77.71	146.51
8	.32	.03	5	.29	145.89
9	94.97	169.61	6	76.88	.28
.810	.62	.20	7	.46	144.66
1	.28	168.79	8	.04	.04
2	93.92	.38	9	75.59	143.35
3	.55	167.92	.860	.14	142.66
4	.18	.46	1	74.68	141.96
5	92.81	.00	2	.23	.26
6	.44	166.53	3	73.79	140.59
7	.07	.07	4	.38	139.96
8	.71	165.62	5	72.96	.32
9	91.36	.18	6	.52	138.65
.820	.00	164.74	7	.09	137.98
1	90.64	.29	8	71.67	.33
2	.29	163.84	9	.25	136.69
3	89.92	.38	.870	70.84	.07
4	.54	162.88	1	.44	135.45
5	.16	.38	2	.04	134.84
6	88.76	161.86	3	69.63	.19
7	.36	.32	4	.21	133.54
8	87.96	160.79	5	68.79	132.89
9	.58	.28	6	.38	.23
.830	.19	159.77	7	67.96	131.58
1	86.81	.26	8	.54	130.92
2	.42	158.74	9	.13	.26
3	.04	.23	.880	66.70	129.57
4	85.65	157.71	1	.26	128.87
5	.27	.19	2	65.83	.19
6	84.88	156.66	3	.42	127.52
7	.48	.10	4	65.00	126.85
8	.08	155.55	5	64.57	.15
.8382	84.00*	.45	6	.13	125.44
.839	83.69	.02	7	63.70	124.73
.840	.31	154.49	8	.26	.02
1	82.92	153.96	9	62.82	123.29
2	.54	.43	.890	.36	122.53
3	.15	152.89	1	61.92	121.79
4	81.76	.34	2	.50	.11
5	.36	151.78	3	.08	120.42
6	80.96	.21	4	60.67	119.74
7	.54	150.61	5	.26	.05
8	.13	.00	6	59.83	118.34
9	79.72	149.38	7	.39	117.61

* Rectified Spirit B. P.

SPECIFIC GRAVITIES OF MIXTURES OF ALCOHOL WITH WATER.—Continued.

Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.	Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.
.898	58.95	116.88	.9330	43.24	89.06
9	.50	.11	35	.00	88.62
.900	.05	115.33	40	42.76	.18
1	57.63	114.62	45	.52	87.73
2	.21	113.92	50	.29	.29
3	56.77	.18	55	.05	86.84
4	.32	112.41	60	41.80	.37
5	55.86	111.64	65	.55	85.90
6	.41	110.84	70	.30	.43
7	54.95	.03	75	.05	84.96
8	.48	109.20	80	40.80	.49
9	.00	108.36	85	.55	.02
.910	53.57	107.61	90	.30	83.54
1	.13	106.86	95	.05	.07
2	52.68	.07	.9400	39.80	82.59
3	.23	105.27	05	.55	.12
4	51.79	104.50	10	.30	81.64
5	.38	103.78	15	.05	.17
6	50.96	.05	20	38.78	80.64
7	.52	102.28	25	.50	.11
8	.09	101.51	30	.22	79.57
9	49.64	100.68	35	37.94	.04
.91984	49.24*	100.00	40	.67	78.50
.9200	.16	99.86	45	.39	77.96
05	48.96	.49	50	.11	.42
10	.73	.08	55	36.83	76.88
15	.50	98.67	60	.56	.34
20	.27	.26	65	.28	75.80
25	.05	97.85	70	.00	.26
30	47.82	.44	75	35.75	74.78
35	.59	.03	80	.50	.30
40	.36	96.62	85	.25	73.81
45	.14	.21	90	.00	.33
50	46.91	95.79	95	34.76	72.87
55	.68	.38	.9500	.52	.41
60	.46	94.97	05	.29	71.94
65	.23	.55	10	.05	.48
70	.00	.14	15	33.76	70.92
75	45.77	93.73	20	.47	.34
80	.55	.31	25	.18	69.76
85	.32	92.89	30	32.87	.16
90	.09	.48	35	.56	68.54
95	44.86	.06	40	.25	67.92
.9300	.64	91.64	45	31.94	.30
05	.41	.23	50	.62	66.68
10	.18	90.81	55	.31	.05
15	43.95	.39	60	.00	65.43
20	.71	89.95	65	30.72	64.87
25	.48	.50	70	.44	.32

* Proof Spirit.

SPECIFIC GRAVITIES OF MIXTURES OF ALCOHOL WITH WATER.—*Continued.*

Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.	Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.
.9575	30.17	63.77	.9684	22.54	48.19
80	29.87	.17	5	.46	.03
85	53	62.49	6	.38	47.87
90	.20	61.82	7	.31	.71
95	28.87	.16	8	.23	.55
.9600	.56	60.53	9	.15	.39
05	.25	59.90	.9690	.08	.23
10	27.93	.26	1	.00	.07
15	.57	58.53	2	21.91	46.92
20	.21	57.80	3	.85	.76
25	26.87	.09	4	.77	.59
30	.53	56.41	5	.69	.43
35	.20	55.73	6	.62	.27
40	25.86	.03	7	.54	.11
45	.50	54.30	8	.46	45.95
.9650	.14	53.56	9	.38	.79
1	.07	.42	.9700	.31	.63
2	.00	.27	1	.23	.47
3	24.92	.11	2	.15	.31
4	.85	52.95	3	.08	.15
5	.77	.80	4	.00	44.99
6	.69	.64	5	20.91	.81
7	.62	.48	6	.83	.63
8	.54	.32	7	.75	.46
9	.46	.16	8	.66	.29
.9660	.38	.00	9	.58	.12
1	.31	51.84	.9710	.50	43.94
2	.23	.69	1	.42	.77
3	.15	.53	2	.33	.60
4	.08	.37	3	.25	.42
5	.00	.21	4	.17	.25
6	23.92	.05	5	.08	.07
7	.85	50.89	6	.00	42.90
8	.77	.73	7	19.91	.73
9	.69	.57	8	.83	.55
.9670	.62	.41	9	.75	.38
1	.54	.25	.9720	.66	.20
2	.46	.10	1	.58	.03
3	.38	49.94	2	.50	41.85
4	.31	.78	3	.42	.68
5	.23	.63	4	.33	.51
6	.15	.47	5	.25	.33
7	.08	.31	6	.17	41.16
8	.00	.15	7	.08	40.98
9	22.91	48.99	8	.00	.81
.9680	.85	.83	9	18.92	.64
1	.77	.67	.9730	.85	.48
2	.69	.51	1	.77	.32
3	.62	.35	2	.69	.16

SPECIFIC GRAVITIES OF MIXTURES OF ALCOHOL WITH WATER.—Continued.

Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.	Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.
.9733	18.62	40.00	.9783	14.50	31.41
4	.54	39.83	4	.42	.22
5	.46	.67	5	.33	.03
6	.38	.51	6	.25	30.84
7	.31	.35	7	.17	.64
8	.23	.19	8	.08	.45
9	.15	.03	9	..00	.26
.9740	.08	38.87	.9790	13.92	.10
1	.00	.71	1	.85	29.93
2	17.91	.53	2	.77	.77
3	.83	.36	3	.69	.61
4	.75	.18	4	.62	.44
5	.66	.01	5	.54	.29
6	.58	37.83	6	.46	.11
7	.50	.66	7	.39	28.95
8	.42	.48	8	.31	.79
9	.33	.31	9	.23	.62
.9750	.25	.13	.9800	.15	.46
1	.17	36.96	1	.08	.29
2	.08	.78	2	.00	.13
3	.00	.61	3	12.92	27.97
4	16.91	.43	4	.85	.80
5	.83	.27	7	.77	.64
6	.75	.11	6	.69	.48
7	.66	35.95	7	.62	.31
8	.58	.77	8	.54	.15
9	.50	.62	9	.46	26.98
.9760	.42	.46	.9810	.39	.82
1	.33	.30	1	.31	.66
2	.25	.14	2	.23	.49
3	.17	34.97	3	.15	.33
4	.08	.82	4	.08	.16
5	.00	.66	5	.00	.00
6	15.91	.50	6	11.92	25.83
7	.83	.32	7	.85	.66
8	.75	.14	8	.77	.50
9	.66	33.96	9	.69	.34
.9770	.58	.78	.9820	.62	.17
1	.50	.61	1	.54	.01
2	.42	.43	2	.46	24.84
3	.33	.26	3	.39	.68
4	.25	.08	4	.31	.52
5	.17	32.91	5	.23	.36
6	.08	.73	6	.15	.20
7	.00	.56	7	.08	.04
8	14.91	.38	8	.00	23.87
9	.83	.18	9	10.91	.67
.9780	.75	31.99	.9830	.81	.47
1	.66	.79	1	.72	.27
2	.58	.60	2	.63	.07

SPECIFIC GRAVITIES OF MIXTURES OF ALCOHOL WITH WATER.—*Continued.*

Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.	Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.
.9833	10.54	22.87	.9882	6.95	15.16
4	.44	.67	3	.89	.03
5	.35	.47	4	.82	14.88
6	.26	.27	5	.75	.73
7	.16	.07	6	.69	.60
8	.07	21.87	7	.62	.45
9	9.99	.70	8	.55	.30
.9840	.92	.55	9	.49	.17
1	.85	.40	.9890	.42	.02
2	.78	.25	1	.35	13.87
3	.70	.08	2	.29	.74
4	.63	20.93	3	.22	.59
5	.56	.78	4	.15	.43
6	.49	.63	5	.09	.30
7	.41	.46	6	.02	.15
8	.34	.31	7	5.96	.02
9	.27	.16	8	.85	12.87
.9850	.20	.01	9	.83	.74
1	.12	19.84	.9900	.77	.61
2	.05	.69	1	.70	.46
3	8.98	.54	2	.64	.33
4	.91	.38	3	.58	.20
5	.84	.23	4	.51	.05
6	.77	.08	5	.45	11.92
7	.70	18.93	6	.39	.79
8	.62	.76	7	.32	.64
9	.55	.61	8	.26	.51
.9860	.48	.46	9	.20	.38
1	.41	.31	.9910	.13	.22
2	.34	.16	1	.07	.09
3	.27	.01	2	.01	10.96
4	.20	17.86	3	4.94	.81
5	.13	.71	4	.88	.68
6	.06	.56	5	.82	.55
7	7.99	.41	6	.76	.42
8	.92	.26	7	.70	.29
9	.85	.10	8	.64	.16
.9870	.78	16.95	9	.57	.01
1	.71	.80	.9920	.51	9.88
2	.64	.65	1	.45	.75
3	.57	.50	2	.39	.62
4	.50	.35	3	.33	.49
5	.43	.20	4	.27	.36
6	.37	.07	5	.20	.20
7	.30	15.92	6	.14	.07
8	.23	.77	7	.08	8.94
9	.16	.62	8	.02	.81
.9880	.09	.47	9	3.96	.68
1	.02	.31	.9930	.90	.55

SPECIFIC GRAVITIES OF MIXTURES OF ALCOHOL WITH WATER.—*Continued*.

Specific Gravity at 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure	Specific Gravity of 60°/60° F.	Percentage of Absolute Alcohol by Weight.	Percentage of Proof Spirit by Measure.
.9931	3.84	8 42	.9966	1.83	4.03
2	.78	.29	7	.78	3.92
3	.73	.18	8	.73	.81
4	.67	.05	9	.67	.68
5	.61	7.92	.9970	.61	.54
6	.55	.79	1	.56	.43
7	.49	.66	2	.51	.32
8	.43	.53	3	.45	.19
9	.37	.40	4	.40	.08
.9940	.32	.29	5	.34	2.95
1	.26	.16	6	.29	.84
2	.20	.02	7	.23	.71
3	.14	6.89	8	.18	.60
4	.08	.76	9	.12	.47
5	.02	.63	.9980	.07	.36
6	2.97	.52	1	.02	.25
7	.91	.39	2	0.96	.12
8	.85	.26	3	.91	.01
9	.79	.13	4	.85	1.87
.9950	.74	.02	5	.80	.76
1	.68	5.89	6	.74	.63
2	.62	.76	7	.69	.52
3	.57	.65	8	.64	.41
4	.51	.52	9	.58	.28
5	.45	.39	.9990	.53	.17
6	.39	.25	1	.47	.04
7	.34	.14	2	.42	0.93
8	.28	.01	3	.37	.82
9	.22	4.88	4	.32	.71
.9960	.17	.77	5	.26	.57
1	.11	.64	6	.21	.46
2	.05	.51	7	.16	.35
3	1.99	.38	8	.11	.24
4	.94	.27	9	.05	.11
5	.89	.16	1.0000	.00	.00

In the part of the foregoing table referring to alcohol of greater strength than proof spirit, only the percentages of alcohol and proof spirit are given which correspond with sp. gr. which can be accurately expressed by three figures. Between the concentrations of 49 and 25% of absolute alcohol the table is more extended, and for still more dilute spirit the percentages of alcohol and proof spirit are given which correspond with every degree of sp. gr. expressed to the fourth place of decimals. As arranged, the table will be found sufficiently

copious for all cases likely to occur in practice. When it is desired to ascertain, in strong spirit, the proportion of alcohol corresponding with a figure of sp. gr. to the fourth decimal place, it may be effected by interpolation. The following example shows the application of the method to a sample of spirit of 0.8673 sp. gr. $72.09 - 71.67 = .42$;

and $\dfrac{.42 \times 3}{10} = .126$; and $72.09 - .126 = 71.96$, as the percentage of alcohol in spirit of 0.8673.

Some forms of pyknometer can be filled at one temperature and weighed after an interval at another, but the ordinary specific-gravity bottle with bored stopper must be weighed immediately it is filled and consequently at the temperature of filling. When, as sometimes happens in a chemical laboratory, the dew-point is above 60° F., the ordinary specific-gravity bottle must be abandoned or the comparison made at a higher temperature, 62° F. or 64° F. or even higher, and, apart from considerations of dew, it is frequently difficult, if not actually impossible, to cool liquids to 60° F. Specific gravities are consequently determined at temperatures higher than 60° F., and, since the coefficient of expansion of alcohol is much greater than that of water, the sp. gr. at 62°/62° is widely different from the sp. gr. at 60°/60°, and a correction must be made. Liverseege (*Analyst*, 1897, **22**, 153) has worked out a table of corrections which represents the mean of the results of several workers, who differ slightly in their recommendations. His table, however, shows the amount to be added to the observed sp. gr. supposing that this has been determined at T°/60°, where T° is some temperature higher than 60°. It is reasonable to suppose that any chemist will determine the water-content of his bottle daily, and that this weighing will be made at the same temperature as that of the aqueous alcohol, and on this supposition the following table has been constructed. It shows the amount to be added to the observed sp. gr. at 61°/61° in order to convert this into the sp. gr. at 60°/60°. If the sp. gr. has been taken at 62°/62°, double the amounts shown in the table must be added to arrive at the sp. gr. at 60°/60°, and so on within reason, but since alcohol expands unequally with equal increments of temperature, the correction is less accurate when applied to temperatures far removed from 60°, and in all alcoholometric work it is well to keep as near to 60° F. as possible.

ADDITIONS TO BE MADE TO SPECIFIC GRAVITIES OF AQUEOUS ALCOHOL OBSERVED AT 61°/61° F.

In Order to Convert them into Specific Gravities at 60°/60° F.

Sp. gr. at 61°/61° F.	Addition.	Sp. gr. at 61°/61° F.	Addition.
0.794 to 0.860	.00039	0.963 to 0.964	.00019
0.860 to 0.890	.00038	0.964 to 0.966	.00018
0.890 to 0.905	.00037	0.966 to 0.967	.00017
0.905 to 0.916	.00036	0.967 to 0.968	.00016
0.916 to 0.925	.00035	0.968 to 0.969	.00015
0.925 to 0.932	.00034	0.969 to 0.970	.00014
0.932 to 0.937	.00033	0.970 to 0.971	.00013
0.937 to 0.941	.00032	0.971 to 0.972	.00012
0.941 to 0.944	.00031	0.972 to 0.973	.00011
0.944 to 0.947	.00030	0.973 to 0.974	.00010
0.947 to 0.949	.00029	0.974 to 0.975	.00009
0.949 to 0.951	.00028	0.975 to 0.976	.00008
0.951 to 0.953	.00027	0.976 to 0.977	.00007
0.953 to 0.954	.00026	0.977 to 0.979	.00006
0.954 to 0.956	.00025	0.979 to 0.981	.00005
0.956 to 0.958	.00024	0.981 to 0.982	.00004
0.958 to 0.959	.00023	0.982 to 0.984	.00003
0.959 to 0.961	.00022	0.984 to 0.986	.00002
0.961 to 0.962	.00021	0.986 to 0.994	.00001
0.962 to 0.963	.00020		

The following rules give the means of calculating percentages of alcohol by weight or volume to the corresponding percentages of proof spirit, and *vice versa*. The percentage of alcohol by volume is a mode of expression not common in England, but is the usual way of valuing spirit adopted in France, Belgium, Germany, the United States and some other countries.

The percentage by *volume of absolute alcohol* may be obtained by multiplying the percentage of proof spirit by the factor 0.5706.

The percentage by *volume of absolute alcohol* may also be obtained by multiplying the percentage of alcohol by weight by the observed sp. gr., and dividing the product by 0.7938 (or multiplying it by 1.26).

The percentage by *volume of proof spirit* can be obtained by dividing the percentage of absolute alcohol by volume by 0.5706 (or multiplying it by 1.7525).

The percentage by *volume of proof spirit* may be obtained by multiplying the percentage by weight of absolute alcohol by the sp. gr., and the product by 2.208.

The percentage of *absolute alcohol by weight* may be found by divid-

ing the percentage of proof spirit by the product of the sp. gr. and 2.208.

The percentage of *absolute alcohol by weight* may be found by multiplying the percentage of alcohol by volume by 0.7938 and dividing the product by the sp. gr.

If the percentage of alcohol by weight be called W, the percentage by volume V, the percentage of proof spirit P, and the sp. gr. D, then the following equations embody the instructions given in the foregoing rules:

$$V = P \times 0.5706.$$

$$V = \frac{WD}{0.7938} = WD \times 1.26.$$

$$P = \frac{V}{0.5706} = V \times 1.7525.$$

$$P = WD \times 2.208.$$

$$W = \frac{P}{D \times 2.208}$$

$$W = \frac{V \times 0.7938}{D}$$

The following are examples of calculations, such as have frequently to be made:

If it be required to know what percentage of gin at 20° U.P. is contained in a watered sample of 44° U.P., the following calculation will suffice:

$$\frac{(100 - 44)100}{100 - 20} = \frac{56 \times 100}{80} = 70 \% \text{ by volume.}$$ Hence the sample is of a strength corresponding to the dilution of 7 gallons of gin at 20° U.P. to 10 gallons by addition of water.

Again, to ascertain the proportion of water which must be added to spirit at 35° O.P., to reduce the strength to 10° U. P.:

$$\frac{(100 - 10)100}{100 \div 35} = \frac{90 \times 100}{135} = 66.7.$$ That is, to obtain spirit of 10° U.P. 66.7 measures of spirit at 35° O.P. must be diluted to 100, or every two gallons must be made up to three by addition of water.

The estimation of alcohol in presence of fixed matters is usually effected by distillation of the sample and ascertaining of the sp. gr. of the distillate. It is sometimes necessary, and generally advisable, to

neutralise the liquid before distillation, but this must not be done when ascertaining the original gravity of beers.

The quantity to be taken will depend on the alcoholic strength of the sample, and is sometimes conditioned by the small quantity supplied to the analyst. 100 c.c. is a convenient quantity of beer or wine. The beer should be distilled till about 80 c.c. has come over, and the distillate should be made up with distilled water to 100 c.c. In the case of wines, it is better to add 50 c.c. of water and a little tannin to 100 c.c. of the wine, and to distil until nearly 100 c.c. has been collected. Of potable spirits, which contaîñ about 50 % of alcohol, it is convenient to mix 50 c.c. with 100 c.c. of water and to distil over about 100 c.c. Stronger spirit should be still further diluted, 25 c.c. being diluted to 150 c.c. with water, and 100 c.c. distilled. The distillate is in any case made up to 100 c.c. and its sp. gr. determined at 60°/60° F. Reference to the tables will at once show the percentage of alcohol by weight contained in the distillate. Then—

$$\frac{\text{Sp. gr. of distillate} \times \text{measure of distillate in c.c.} \times \%\text{of alcohol found in distillate by table}}{\text{Sp. gr. of sample} \times \text{measure of sample taken in c.c.}}$$

= Percentage of absolute alcohol by weight contained in *the sample*.

This calculation involves the necessity of knowing the sp. gr. of the *original sample*. If unknown, the 50 or 100 c.c. taken for the experiment may be accurately weighed, and this weight in grams substituted for the denominator of the above fraction.

The calculation can be wholly avoided, and a more satisfactory result obtained by *weighing* the original sample instead of measuring it, and also weighing the distillate. Then—

$$\frac{\text{Weight of distillate} \times \text{percentage of alcohol found in distillate by table}}{\text{Weight of sample taken}}$$

= Percentage of absolute alcohol by weight contained in *the sample*.

The following *indirect method* is less accurate than the distillation method, but is sometimes useful. The sp. gr. of the original liquid is first taken, and then 100 c.c. is evaporated to expel alcohol and subsequently made up again with distilled water to 100 c.c. at 60° F., and the sp. gr. of the alcohol-free liquid determined. Let this be S_2 and the sp. gr. of the original liquid S_1. From these numbers it is possible to calculate with fair accuracy what would have been the sp. gr., S, of the distillate, supposing the liquid had been distilled and the distillate made up to 100 c.c., as follows:

$$S = 1 + S_1 - S_2.$$

From the value of S thus found, the percentage of alcohol may be found by reference to the tables already given.

The alcoholic strength of *potable spirits*, which, with the exception of gin, rarely contain more than 0.5% of solid matter, may be approximately ascertained from the sp. gr. of the original spirit and the proportion of solid matter. If the spirit has a sp. gr., S_1, and contains W grm. of solid matter per 100 c.c., then—

$$S = S_1 - 0.0055W,$$

where S has the same significance as in the last paragraph.

The *refractometer* may be applied to the estimation of alcohol in liquids consisting solely of alcohol and water. In the presence of fixed matters, resort must be had to distillation, or to an indirect method exactly analogous to the indirect sp. gr. method. A table for use with the Zeiss immersion refractometer has been already given in the section on Methyl Alcohol. A more extended one is that of Wagner and Schultze (*Zeits. anal. Chem.*, 1907, 46, 508), and Wagner, who has from time to time, in the *Chemiker Zeitung* and elsewhere, published sections of the table in greatly extended form, with convenient temperature corrections for special purposes, has recently brought out a book (*Tabellen zum Eintauchrefraktometer;* Carl Zeiss, Jena, 1903) to which readers are referred for further information. The refractometer is particularly useful in estimating methyl and ethyl alcohols in admixture, but for the estimation of ethyl alcohol in aqueous solution it is a less accurate instrument than the sp. gr., and if it is desired to obtain results of any value by its means, so much attention must be paid to the temperature that its use can hardly be recommended on the score of speed.

Boiling-point Method.—The estimation of the proportion of alcohol in a liquid may be made by noting the temperature of the vapour given off from the boiling liquid. Wiley has described a form of apparatus for this purpose (*Jour. Amer. Chem. Soc.*, 1896, 18, 1063), which he claims yields quite accurate results. It consists of the flask, F, which is closed by the rubber stopper, carrying the large thermometer, B, and a tube leading to the condenser, D. The vapours which are given off during ebullition are condensed in D and return to the flask through the tube, as indicated in the figure, entering the flask below the surface of the liquid.

The flask is heated by a gas-lamp and is placed upon a circular disc

of asbestos in such a way as to cover entirely the hole in the centre of the asbestos disc, which is a little smaller than the bottom of the flask. The whole apparatus is protected from external influences of temperature by the glass cylinder, E, which rests upon the asbestos disc below and is covered with a detachable stiff rubber-cloth disc above.

The thermometer, C, indicates the temperature of the air between F and E. The reading of the thermometer, B, should always be made at a given temperature of this surrounding air. The tube leading from the condenser, D, to the left is made long and is left open at its lower extremity in order to maintain atmospheric pressure in F and at the same time prevent the diffusion of the alcoholic vapours through D.

The flame of the lamp is so regulated as to bring the temperature indicated by the thermometer C to about 90° in ten minutes for substances containing not over 5% of alcohol. After boiling for a few minutes, the temperature, as indicated in the thermometer B, is constant, and the readings of the thermometer should be made at intervals of about half a minute for ten minutes. Some pieces of scrap platinum placed in the flask will prevent bumping and secure a more uniform evolution of vapour. Slight variations due to the changes in temperature of the vapours are thus reduced to a minimum effect upon the final results. The apparatus is easily operated, is quickly charged and discharged and with it at least three assays of alcohol can be made in an hour.
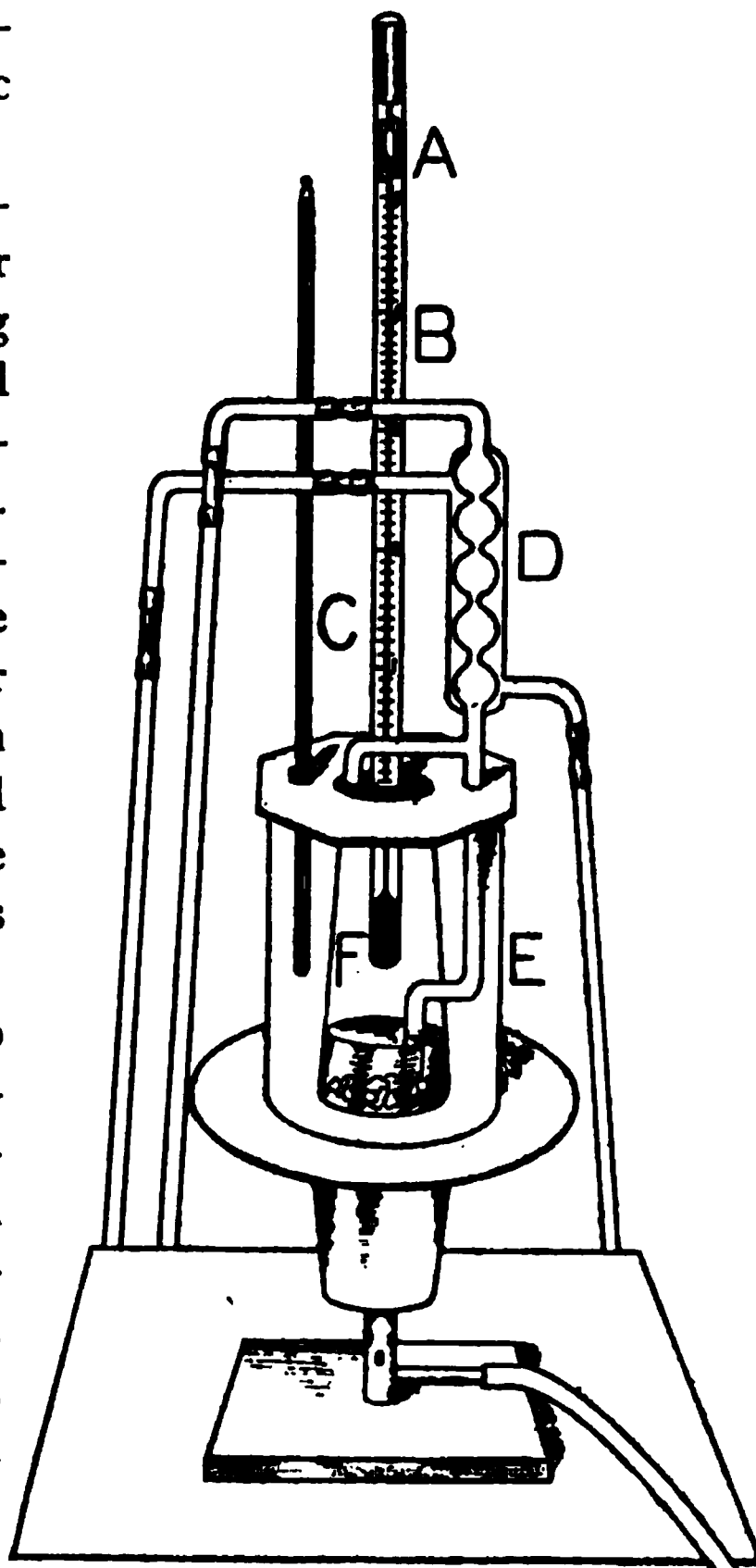
FIG. 52.

The thermometer used is the same that is employed for the indication of freezing- and boiling-points in the estimation of molecular weights. The reading of the thermometer is arbitrary, but the degrees indicated are Centigrade. The thermometer is set in the first place by putting the bulb in water containing 16 grm. of common salt to 100 c.c. when the water is fully boiling, the excess of mercury is removed from the column in the receptacle at the top, and then, on placing in boiling water, the column of mercury will be found a little above the 5° mark. This will allow a variation in all of 5° in the temperature, and a thermometer thus set can be used for the estimation of percentages of alcohol from 1 to 5.5 by volume. When the liquor contains a larger percentage of alcohol than this, it is advisable to dilute it until it reaches the standard mentioned.

In order to avoid frequent checking of the thermometer, rendered necessary by changes in barometric pressure, a second apparatus, made exactly like the one described, is used, in which water is kept constantly boiling. It is only necessary in this case to read the two thermometers at the same instant in order to make any necessary correction required by changes in barometric pressure.

While no table showing the percentages of alcohol corresponding to any given depression in the temperature of the vapour is appended, attention is called to the fact that the plotted line showing the variation in depression of zero to 5% by volume of alcohol is practically straight, and that for each 0.8° change in temperature of the vapour there is a change of about 1% by volume of alcohol. This rule can be safely applied for practical purposes to all liquors contain ng not more than an 5.5 % of alcohol. For example, if, in a given case, the temperature of the vapour of boiling water, as marked by the thermometer, is 5.155° and the temperature of that from a sample of beer is 2.345°, the depression is equivalent to 2.810, and the percentage of alcohol by volume is, therefore, 2.81 divided by 0.80 = 3.51.

The thermometer used is graduated to hundredths of a degree, and, read by means of a cathetometer, will easily give readings to five thousandths of a degree.

The reading of the thermometer is facilitated by covering the bulb with a test-tube containing water. The high specific heat of the water distributes evenly any little variations of temperature which otherwise would cause the mercurial column in thermometer B to oscillate. The water jacket also serves as a protection against the

projection of any particles of the boiling liquor directly against the bulb of the thermometer.

Freezing-point Method.—Alcohol in aqueous solution can be estimated with fair accuracy by observing the depression of the freezing-point in Beckmann's apparatus, provided the alcohol does not exceed '7%. Below that strength, the depression of the freezing-point is approximately proportional to the percentage of alcohol, being being, according to Gaunt (*Zeit. anal. Chem.*, 1905, 44, 106), 0.425 for each 1 %. The method is said by Gaunt to be quicker than the sp. gr. method.

Other methods for the estimation of alcohol have been based on its rate of dilatation by heat, on the surface-tension of the liquid, and on the tension of its vapour. These methods are capable of being used with advantage under special circumstances, but they require special apparatus and are generally less accurate and convenient than those already given.

The Estimation of Alcohol in Essences, Tinctures and other preparations containing substances volatile with alcoholic steam presents difficulties that may in most cases be surmounted by having recourse to the following method, due to Thorpe and Holmes (*Trans. Chem. Soc.*, 1903, **83,** 314):

"25 c.c. of the sample, measured at 60° F., are mixed with water in a separator to a bulk of from 100 to 150 c.c., and sodium chloride is added in quantity sufficient to saturate the liquid. The mixture is now shaken vigourously for 5 minutes with from 50 to 80 c.c. of petroleum spirit, boiling below 60° C., and after standing for about half an hour, the lower layer is drawn off into another separator, extracted, if necessary, a second time with petroleum spirit, and then drawn off into a distillation flask. Meanwhile the layers of petroleum spirit, are washed successively with 25 c.c. of saturated sodium chloride solution, and the washings added to the main bulk, which is neutralised if necessary, and then distilled and the distillate made up to 100 c.c.

"The method, as described, is applicable to preparations containing ether, chloroform, benzaldehyde and esters. In the greater number of cases, for example, essences of lemon, juniper, peppermint and santal-oil preparations, a single extraction is sufficient.

"In the case of all preparations containing camphor, 25 c.c. of normal sulphuric acid are used instead of sodium chloride, and one extraction spirit is made. Before distilling it is desirable to neutralise

with sodium hydroxide, and if the volume of . the liquid becomes inconveniently large some sodium chloride is also added. In preparations containing ammonia this is inadmissible, and the liquid to be distilled must be slightly acid.[1]

The Estimation of Ethyl Alcohol in Fusel Oil is sometimes required, since fusel oil containing less than 15 % of proof spirit is admitted duty free into the United Kingdom. Thorpe and Holmes have shown that the method, above described, enables them to estimate the ethyl alcohol accurately in a mixture of 73 alcohol, 20 fusel oil and 7 water. If the proportions are 8 alcohol, 90 fusel oil and 2 water, it may be necessary to use rather more petroleum spirit and less sodium chloride solution, but otherwise the method is presumably applicable.

Formerly fusel oil was tested by the Excise by shaking it with an equal volume of water to remove the spirit, and then ascertaining the amount of alcohol contained in the aqueous liquid by taking its sp. gr. and noting its volume. The test gives erroneous results, as fusel oil is a mixture of alcohols, of which only amyl alcohol is approximately insoluble in water. As an improvement on this test, G. L. Ulex (*Neues Jahrb. der Pharm.*, **39**, 333) recommended the following, based on the low temperature at which ethyl alcohol distils; 100 c.c. of the sample are heated in a retort until 5 c.c. have passed over; the distillate is shaken with an equal volume of a saturated solution of sodium chloride, and the mixture allowed to stand. If the fusel oil which separates amounts to one-half of the distillate or more, the

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[1] The requirement of the U. S. (Federal) food-law and of some state food-laws, that medicinal preparations must bear on the label a statement of the percentage of alcohol present renders it necessary for manufacturers to verify the amount in preparations when finally prepared for sale. C. E. Vanderkleed (*Amer. Jour. Pharm.* 1909, **89**, 129) has made a special study of this phase of the problem and recommends the following method:

50 c.c. of the preparation, measured at a known temperature, are transferred (in portions, if necessary) to a test tube having an inside diameter of 22 mm. and a height of 200 mm., marked at 50 c.c. The tube is heated until in the water bath all alcohol is driven off. The liquid is cooled to the original temperature, and U. S. Pharmacopœia alcohol (see page 112) at the same temperature is run in from a burette until an amount has been added which when diluted with water to exactly 50 c.c. would give the same alcoholic strength as the menstruum that was used in manufacture of the preparation being assayed. The tube is stoppered, the contents mixed and the sp. gr. ascertained (Vanderkleed uses a Westphal balance). Subtract algebraically the original sp. gr. from that of the solution obtained in the process, and subtract this remainder from the theoretical dilution above noted, and ascertain from the standard tables the percentage of alcohol.

Vanderkleed assayed fluid extracts of Buchu, Cubeb and Santal of known composition and found that the simple distillation method and the method of Thorpe and Holmes gave lower results than the above method. Inasmuch as these complex medicinal preparations are liable to furnish a distillate that contains other substances than alcohol, Vanderkleed does not regard the refraction method as offering any advantage in the solution of this special problem.—H. L.

sample is sure to contain less than 15 % of spirit, and is free from any fraudulent admixture with the same. If less fusel oil, or none at all, separates, the presence of 15 % of the spirit may be safely assumed. In the latter case, the quantity of the adulterant may be ascertained by shaking a known measure of the sample with an equal bulk of a saturated solution of sodium chloride (in which propyl and butyl alcohols are much less soluble than in water), allowing the aqueous liquid to settle out, distilling it, and estimating the contained alcohol by noting the volume and sp. gr. of the distillate.

Allen showed the accuracy of another method of approximately separating amyl alcohol from ethyl alcohol, which is to agitate the sample in a graduated tube with an equal volume of benzene or petroleum spirit, subsequently adding sufficient water to cause the benzene to separate. The increase in the volume of the benzene indicates with approximate accuracy the amount of amyl alcohol in the sample under examination.

Peters (*Pharm. Centralh.*, 1905, 46, 563) has described a method, similar to that of Thorpe and Holmes, but more complicated. The fusel oil is shaken with water and petroleum spirit and the aqueous layer distilled. The distillate is then shaken with light petroleum spirit and calcium chloride solution and the aqueous layer again distilled.

MALT AND MALT LIQUORS.

By JULIAN L. BAKER, F.I.C.

Malt is prepared by steeping barley or other grain in water, and allowing it to germinate, the sprouted grain being subsequently dried and cured in a kiln. During these operations the composition of the grain is materially modified. There is a reduction in the amount of starch which has been used up by the growing embryo and an increase in the soluble carbohydrates; also a large quantity of the insoluble nitrogenous matters present in the barley becomes converted into soluble modifications. The following figures % illustrate the differences between barley and malt (English).

	BARLEY. per cent.	MALT. per cent.
Moisture	15	2
Proteins	10	11
Fat	2	0
Sugar and gum	11	16
Starch	55	63
Fiber	5	6
Ash	2	2

Well-malted barley ranges in colour from light to dark yellow according to the origin of the barley and the degree of curing. On breaking the malt corn the interior should be pure white, unless the drying has been intentionally carried so far as to partially caramelise the sugar, as, for example, with amber malts. It is customary for the brewer or maltster to form an opinion of a sample of malt from its crispness and flavour.. Each corn should break easily between the teeth and the sweet characteristic malty flavour should be quickly developed. If the corns are hard or steely it indicates that the drying has been improperly carried out, the resulting high temperature having vitrified the corns, or the acrospire has not been sufficiently grown with the result that the grain is not properly modified. Malt should be free from broken and damaged corns or culms (dried rootlets).

Chemical Examination of Malt.—The brewing value of a sample of malt is chiefly dependent on 3 factors, namely, the proportion of soluble or extractive matter it will yield to water; the character of this extractive matter; and the diastatic activity.

The proportion and composition of the extractive matter are influenced by many conditions, including the temperature of the water used for mashing, the character of the water, the proportion employed, the composition of the original malt, and the temperature at which it is dried.

In 1905 the Council of the Institute of Brewing (*J. Inst. of Brewing*, 1906, **12,**) appointed a committee to report upon suitable methods for estimating the extract, moisture, diastatic power, colour, and percentage of ready-formed sugars in malt. As these procedures are now usually carried out in all English laboratories associated with the malting or brewing industries they may be regarded, at any rate for the present, as "standard methods."

Sampling.—It is obvious that samples sent for analysis should, so far as possible, be fairly representative of bulks, and this requires the more care when the bulks are large, and when the malt contains any appreciable number of hard corns, and further, when there is any marked irregularity in curing.

In the case of deliveries, samples should be drawn from at least one sack in every 10 if the consignment amounts to over 100 sacks, or if the parcel be smaller, then from 10 % of the number of sacks. The sample should be withdrawn not from the surface of a sack, but from a depth of at least six inches.

These bulk samples should be put into a small bin or other suitable receptacle, thoroughly well mixed up and the requisite number of samples collected in clean screw-stoppered beer bottles.

If uniform results are to be obtained it is essential that the grists should be uniform; accordingly the committee advised that the Seck Mill set at 25° should be used. In order to allow for loss in the mill, a quantity of malt, slightly in excess of that required for each determination should be separately weighed and ground. Finally the exact amounts of grist, subsequently required for the various determinations, are weighed out. It is not permissible to grind at the outset sufficient malt for all the procedures and to weigh the various quantities from this grist.

Extract.—50 grm. of ground malt are mashed in a glass beaker of

about 500 c.c. capacity with 360 c.c. of distilled water previously heated to 154° to 155° F. The beaker is covered with a clock glass, and placed in a water-bath, so that its contents are kept at a temperature of 150° F. for 55 minutes. The mash is stirred at intervals of about 10 minutes during this time. The temperature is then raised to 158° F. in 5 minutes, and the whole mash washed into a flask gradu-ated to 515[1] c.c., cooled to 60° F., made up to the mark with distilled water at the same temperature, well shaken and filtered through a large-ribbed paper. The sp. gr. of the filtrate is then determined at at once at 60° F., compared with water at that temperature.[2]

If preferred, the mashing can be carried out directly in the 515 c.c. measuring flask. In this case the mash should be shaken at intervals of about 10 minutes.

Colour of Wort.—For this determination the Lovibond tintometer is employed. The above wort, filtered perfectly bright, should be placed at once in a 1-in. cell, and its tint recorded in colour units of the series "52" glasses. The experiment should not be carried out in direct sunlight, and the light must fall equally on both halves of the white plate so that both fields—viz., the malt-extract field and the standard field—are equally illuminated. To test this, the glasses and the cell should be reversed, and all results rejected when the figures do not agree, whichever side the cell is placed.

J. L. Baker and H. F. E. Hulton (*J. Inst. of Brewing*, 1906, **12**, 302; *ibid.*, 1907, **13**, 26) have drawn attention to the discrepancies which arise in reading the colour of worts and beers in the Lovibond tintometer when the position of the instrument is varied relatively to the illumination. It is recommended that the tintometer, in a horizontal position, be directed to a north window, covered with a piece of thin white tissue-paper, and that the opal screen provided with the instrument be dis-carded. J. W. Lovibond (*ibid.*, 1908, **14**, 2) has recently devised a standard lamp which claims to overcome the attendant difficulties of daylight as a standard source of illumination.

Moisture.—About 5 grm. of ground malt are weighed out in a shallow copper vessel, about 5 cm. in diameter and 1.25 cm. in depth,

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[1] The grains from 50 grm. of malt are supposed to occupy a volume of 15 c.c.
[2] In warm weather it is inconvenient to weigh a specific-gravity bottle containing a liquid at a temperature of 60° F.; 65° or 70° F. are more suitable temperatures. It has, however, been pointed out by G. C. Jones (*J. Inst. of Brewing*, 1908, **14**, 9) that it is not sufficient to determine the weights of malt extract and water contained by the pyknometer at the same temperature. The results so obtained must be corrected for the difference in the coeffi-cients of expansion. At 65° F. 0.2 must be added to the brewer's pounds, and at 70° F. 0.5. The excess sp. gr. over water (—1,000) multiplied by 3.36 gives the extract in brewers' pounds per standard quarter of malt.

and kept for 5 hours in a boiling water oven, allowed to cool in a desic-
cator, and reweighed, the loss in weight being taken as the moisture
content and calculated as a percentage on the malt.

Diastatic Activity (Lintner Value).—The measurement of dias-
tatic activity is based on Kjeldahl's law of proportionality (*Compt.
rend. des travaux du laboratoire de Carlsberg*, 1879, **1**, 109; *vide* also
A. R. Ling, *J. Fed. Inst. Brewing*, 1896, **2**, 335). When working with
malt diastase Kjeldahl found that if the production of maltose does not
exceed 45 % of the starch used, this maltose may be taken as a measure
of the diastatic activity of the solution.

25 grm. of ground malt are extracted with 500 c.c. of distilled water
for three hours at 70° F.[1] and filtered. The first 100 c.c. of the
filtrate is rejected. 3 c.c. of the perfectly bright extract are allowed to
act on 100 c.c. of a 2 % solution of soluble starch at 70° F. for an hour
in a 200 c.c. flask.

Preparation of Soluble Starch.—Purified potato starch is digested with dilute
hydrochloric acid, sp. gr. 1,037, at the room temperature (60° to 65° F.) for seven
days, stirring the mixture daily. 500 grm. of starch and 1,000 c.c. of dilute acid
being suitable quantities. The mass is washed very thoroughly by decantation,
at first with tap water and later on with distilled water, until the wash water is free
from acid. It is collected on a filter-paper placed in a Buchner's funnel, pumped as
dry as possible, and then spread out on a new unglazed plate. The starch should
be dried at a gentle heat (110° F.) as quickly as possible. When dry, the starch is
triturated in a porcelain mortar and rubbed through a fine hair sieve.

Starch Solution.—In determining diastatic capacity, the starch must be dis-
solved in boiling water at the rate of 2 grm. of the starch per 100 c.c. of water; the
solution is then cooled to 70° F. for use. It should be mobile (not gelatinous),
indicating perfect conversion into soluble starch, and showing only a negligible
reducing action on Fehling's solution; and it should be neutral to litmus solution.

N/10 alkali (10 c.c.) is then added in order to stop further diastatic
action, the liquid cooled to 60° F., made up to 200 c.c. with distilled
water at the same temperature, well shaken, and titrated against 5 c.c.
portions of Fehling's solution, using ferrous thiocyanate as indicator.

The method of titration, which was devised by A. R. Ling is carried
out as follows:

5 c.c. of Fehling's solution (see p. 318) are accurately measured
into a 150 c.c. boiling flask, and raised to boiling over a small naked
bunsen flame. The converted starch solution is added from a burette,
in small quantities at first of about 5 c.c., the mixture being kept

[1] The water used for this extraction and also for the preparation of the starch solution
must be free from ammonium compounds nitrites and other impurities which may in-
fluence diastatic conversion. The water should be redistilled with the addition of a little
potassium permanganate and sodium hydroxide until the distillate is pure and neutral to
litmus solution. G. C. Jones (*J. Inst. of Brewing*, 1908, **14**, 12) finds that alizarin paste
(1 grm. in 200 c.c.) is a more satisfactory indicator than litmus.

rotated and boiled after each addition until reduction of the copper is complete, which is ascertained by rapidly withdrawing a drop of the liquid by a glass rod and bringing it at once in contact with a drop of the indicator on a porcelain or opal glass slab.

The results are calculated by the following formula:

$$A = \frac{1000}{x\,y}$$

in which A equals the diastatic activity, x, equals the number of cubic centimetres of malt extract contained in 100 c.c. of the fully diluted starch conversion liquid, and y equals the number of cubic centimetres of the same liquid required for the reduction of 5 c.c. of Fehling's solution.

The above method (using 3 c.c. of malt extract to 100 c.c. of 2 % soluble starch solution) is not accurate for malts having a diastatic capacity exceeding 50 Lintner; in the case of such malts the relative volume of malt extract must be less, say 2 c.c., or, for malts of the highest diastatic capacity, such as are frequently used by distillers and vinegar makers (*i.e.*, malts of a diastatic power of over 80° Lintner), an even smaller volume of extract must be taken.

An alternative method which is largely employed consists in measuring 10 c.c. of a 2 % solution of soluble starch into each one of a series of eight carefully cleaned test-tubes. The tubes and their contents are then placed in a suitable stand and immersed in a water-bath at a temperature of 70° F. As soon as the starch solution has reached this temperature 0.1 c.c. of the malt extract (prepared as before) is measured into the first of the tubes. The second tube receives 0.2 c.c., the third 0.3 c.c., and so on until the eight test-tubes contain malt extract in regularly increasing quantities. The tubes are replaced in the stand and immersed in the water bath at 70° F. for exactly one hour from the time the malt extract was added to the first tube. To each tube is then added 5 c.c. of Fehling's solution; after shaking, the tubes are heated in a boiling-water bath for ten minutes and allowed to stand until the cuprous oxide has settled. It will usually be noticed that the liquid of one tube in the series is faintly blue, showing that there was insufficient maltose formed to reduce the copper sulphate, whilst the succeeding one is yellow due to over-reduction. If, for example, tube 3 was under-reduced as much as tube 4 was over-reduced, the reading would be taken as 0.35 c.c. Intermediate points are judged by inspection. Sometimes the solution in one of the tubes will be neither blue

nor yellow, showing that the amount of maltose formed was just enough for complete reduction. If 0.1 c.c. of malt extract corresponds to a diastatic power of 100 and x equals the quantity of malt extract added to the tube, the contents of which occasioned or were adjudged to occasion complete reduction of the Fehling's solution, then the diastatic power of a sample of malt may be calculated from the expression:

$$\frac{0.1 \times 100}{x}$$

It is customary to deduct 1.5 from the diastatic power found, owing to the reducing sugars present in the malt extract. When highly diastatic malts are examined the malt extract should be diluted with an equal volume of water and the reading obtained doubled.

Cold Water Extract.—25 grm. of ground malt are digested with 250 c.c. of distilled water containing 20 c.c. of N/10 ammonium hydroxide (*i.e.*, 20 c.c. of N/10 ammonium hydroxide made up to 250 c.c. with distilled water) for three hours at 70° F., stirring about three or four times during this period. After filtering, the sp. gr. of the bright filtrate is taken at 60° F., compared with water at the same temperature. The excess sp. gr. over water (= 1,000), corrected for the sp. gr. of the ammonium hydroxide, divided by 3.86 and multiplied by 10 gives the cold water extract per cent.

Considerable importance is still attached by many brewing chemists to the significance of the percentage of matter soluble in cold water, as it is claimed to be an indication as to whether a malt has been properly made. If the growth of the sprouted barley is unduly hastened on the malting floor (forcing) more of the starch is converted into sugar and more insoluble matter into soluble matter than if the growth had been slow. Wet loading on the kiln will also occasion an increase in matters soluble in water. In recent years doubts have been expressed as to the value of this datum, for it is by no means proved that a so-called "forced malt" will of necessity produce an unsound beer.

The soluble matters consist of proteins, ash, acid and "ready-formed carbohydrates" (cane-sugar, invert sugar and maltose). An average figure for an English malt is 18 %, half of which is due to carbohydrates.

Statements of Results.—The results, expressed to the nearest first decimal place only, except in the case of *diastatic activity*, which

should be recorded only to the nearest integer, are usually set out as follows:

Extract per standard quarter, brewers' pound,
Moisture, per cent.,
Diastatic activity (Lintner value),
Tint (10 % wort, 1 in. cell, "52" series Lovibond),
Cold water extract per cent.,

In addition to the estimations described above, the following afford information of a useful character.

Acidity.—The acidity of a malt is calculated as lactic acid, which, however, is incorrect, as the acid reaction of most malts is due chiefly to acid phosphates. The estimation, the value of which is entirely empirical, is made by digesting 50 grm. of ground malt with 300 c.c. of distilled water at 60° F. for 3 hours and measuring the acidity of the filtered extract by means of $N/20$ ammonium hydroxide, using litmus paper as an indicator.

Modification.—Malts differ considerably in the extent to which modification has taken place. If the growth has been insufficient on the floor the finished malts will have steely ends, and these will not yield the full extract when mashed, as the starch will not be amenable to the action of diastase. Modification may be conveniently measured by mashing a fine and coarse grind of the same malt under the conditions previously given. In a well-made malt the extracts of a fine and coarse grind will be practically the same, in steely malts the differences will be considerable (3 to 4 pounds per quarter).

Nitrogenous Constituents.—The total amount of nitrogenous matter in the malt (10 to 11%) is determined by Kjeldahl's method and the nitrogen figure found multiplied by 6.25. The insoluble nitrogenous matter is the difference between the total nitrogenous matter and that soluble in water Malt contains less nitrogenous matter than the barley from which it was made owing to the loss during germination. The soluble nitrogenous matter in barley is about 5%; in malt 2.5%.

The "Saccharification" Test.—This test has been devised to measure the time required for the complete saccharification of a malt mash. 10 grm. of the ground malt are mixed with 100 c.c. of water at 154° F. and kept at 151° F. in a suitable bath, the mash being stirred occasionally. In 15 minutes about 5 c.c. of the mash are withdrawn, filtered and the cooled filtrate tested for the presence of starch by iodine. If starch is found the test is repeated at intervals of 5 minutes until the

iodine reaction is no longer observed. The time taken for the complete saccharification is then noted. (A. J. Brown, *Laboratory Studies for Brewing Students*, 62.)

Dry Grains.—The concentration of the wort in the extract estimation is arrived at by dividing the excess gravity above 1000 by 4. As the proportion of malt used to water was as 10:100, the dry extract multiplied by 10 represents the dry extract in 100 grm. of malt. The percentage of dry extract subtracted from 100 gives the percentage of dry grains. The result is, of course, corrected for the moisture in the malt.

PHYSICAL EXAMINATION.

Growth.—100 or preferably 200 malt corns are counted and sorted into the following six groups according to the development of the acrospire, 0 to 1/4; 1/4 to 1/2; 1/2 to 3/4; 3/4 to 1; overgrown, and damaged corns. The length of the acrospire is conveniently observed by feeling the husk of the malt corn on the round side beginning at the germ end. In well-made English malt 80 to 100% of corns are from 3/4 to fully grown.

The "Sinker" Test.—500 corns are counted and stirred into a beaker containing cold water. The corns which float are removed. Some corns will lie flat on the bottom of the beaker, and when these are examined they will be found to be either ungerminated barley, very steely, or vitreous corns; other corns may rest on one end and these will probably be steely-tipped.

Malt should be free from impurities, such as stones, (which frequently cause explosions during the grinding) dirt, or foreign seeds. Serious arsenical contamination now rarely occurs. If present in quantity (a safe limit is 1/300 grain of arsenous oxide per pound) it may usually be traced to carelessness on the part of the maltster, such as mixing gas coke with the anthracite. For the estimation of arsenic in malt and beer, see page 146.

MALT WORTS.

Total Solid Matter.—The sp. gr. of a malt wort is ascertained in the laboratory by a sp. gr. bottle, and this figure minus 1,000 (water = 1,000) and divided by 4 gives the number of grm. of solid matter (dry extract) contained in 100 c.c. of the wort.

For the purposes of the brewer the sp. gr. of the wort may be ascertained by the hydrometer, various modifications of which have been devised for this purpose.

Bates' brewers' saccharometer is an instrument the indications of which are expressed in "pounds per barrel," and these may be translated into absolute gravities by dividing the number of "saccharometer pounds" by 0.36 (or multiplying by 2.778) and adding 1000. A barrel (=36 gallons) of water weighs 360 pounds; a beer-wort, a barrel of which weighs 380 pounds (=360+20), is said to have a "saccharometer gravity of 20 pounds per barrel." The real sp. gr. of such wort would be 1055.5;—for 360:380=1000:1055.5; and it would contain 13.8 grm. of solid extract per 100 c.c. or 50.1 pounds per barrel of 36 gallons. Similarly, a wort of 1055 sp. gr., which is the standard strength of beer wort on which the duty of 6s. 9d. per barrel is levied, has a saccharometer gravity of 20.52 pounds per barrel; for 1055—1000=55; and 55 X 0.36=19.80.

Corrections of sp. gr. of beer worts for temperature can be made as described on page 123.

• The method of ascertaining the original gravity of malt or beer worts which have undergone fermentation is described on page 151.

The solid matter of malt worts consists of a mixture of dextrins, sugars, nitrogenous matters and ash constituents. The work of O'Sullivan, Brown and Morris, Lintner, Ling and many others has shown how very complicated are the products formed by the action of diastase on starch.

In the mash tun, maltose, a series of dextrins differing in molecular weights and complexity (the so-called malto-dextrins), probably dextrose and the preexisting carbohydrates in the malt are present. To follow the nature of the conversion in the mash tun by fully analysing the wort involves an amount of work which, in the opinion of the writer, is not warranted by the results obtained.[1]

Useful data for control purposes are furnished by the specific rotatory power and cupric reducing power of the wort and from these the percentage of apparent maltose and apparent dextrin on the wort solids may be calculated.

Estimation of "Apparent Maltose and Dextrin."—The wort is boiled to throw out any coagulable proteins, filtered and the sp. gr.

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[1]Moritz and Morris describe an elaborate scheme for the analysis of worts in *Text-Book of the Science of Brewing.*

at 15.5/15.5°. This figure minus 1,000 and divided by 4 will give the grm. of wort solids per 100 c.c.

Specific Rotatory Power.—A wort light in colour may be read directly in a 100 or 200 mm. tube. If the wort should be dark it may be clarified with basic lead acetate or alumina cream as in the case of raw sugars. Black beer worts require a special treatment, as basic lead acetate will not remove all the colour. Heron has suggested treating the wort with bleaching powder, but highly caramelised worts are not always sufficiently decolourised by these means for reading in a polarimeter. The writer has found that any black wort may be decolourised with phosphotungstic acid. . The reagent is prepared by dissolving phosphotungstic acid in water and adding 20 % sulphuric until there is a slight turbidity. To 25 c.c. of the original black wort 4 c.c. ofphosphotungstic acid are added and 10 c.c. of 20 % sulphuric acid. The contents of the flask are made up to 100 c.c. filtered and read.

Reducing Power.—For this determination the conditions advised by Brown, Morris and Millar are very commonly employed. However, for rapid and accurate work, the volumetric process as modified by. Ling and Rendle (*Analyst*, 1908, **33**, 167, see also page 136) may be recommended, and if carried out under standard conditions the results are probably quite as accurate as the gravimetric method (Ling and Jones, *ibid.*, 1908, **33,** 167).

For the purpose of the calculation the specific rotatory power of maltose is taken as 137 and dextrin as 200. All reducing sugar present is supposed to be maltose. The grm. per 100 c.c. of maltose $\times$ 1.37 = rotation due to maltose. The total reading minus that due to the "apparent" maltose divided by 2.00 = dextrin in grm. per 100 c.c.

The percentage of "apparent maltose" in mash tun worts calculated on the solids varies between 70 and 80, the "apparent dextrin" between 4 and 10.

ROASTED MALT AND BARLEY.

These materials are used principally in the brewing of stouts; small quantities are sometimes added to a pale malt grist for the purpose of adjusting the colour of a beer. For technical control purposes it is customary to determine extract, colour and moisture.

Colour.—3 grm. of the finely divided material are mixed with 300 c.c. of distilled water at a temperature of 165° F., allowed to stand

ten minutes and the solution read in a 1/4 in. cell in the Lovibond tintometer and the resulting figure multiplied by 2. (J. L. Baker and H. F. E. Hulton, *J. Inst. Brewing*, 1907, 13, 32.) A roasted barley should have a colour of at least 60°. Roasted malts are frequently slightly higher.

Extract.—25 grm. each of roasted barley or malt and a pale malt of a diastatic power not exceeding 30° are mashed together under the conditions described previously. A mash of the pale malt is made simultaneously. The gravity is deetrmined of the extracts and that due to the pale malt subtracted from the extract of the mixture. The difference multiplied by 2 represents the extract yielded per quarter (336 pounds) of the roasted malt or barley. The extract figure varies according to the class of barley or malt roasted and the degree of roasting. With a colour of 60° the extract may vary between 75 and 90 pounds per quarter of 336 pounds.

Moisture is estimated in the same manner as a malt.

Roasted barley is now largely taking the place of roasted malt, the latter being used mostly in the brewing of export stouts. Since roasted malt is more expensive than roasted barley, it is necessary to see that the former when ordered is delivered. Usually this can be done by observing if the acrospire shows any sign of development. The lower nitrogen content of roasted malt as compared with barley has been proposed as a means of differentiation, but the wisdom of estimating nitrogen in bodies which have been submitted to such high temperatures as to be charred is doubtful. There are other kinds of semi-roasted malt used in brewing, such as crystal malt, brown malt, etc. They may be analysed in the same way as roasted barley. The colour should be read in a 1 in. cell.

MALT SUBSTITUTES.

In recent years many of these preparations have been placed on the market. Most of them are derived from maize or rice. The starch in the grain is rendered amenable to diastatic action by being submitted to a torrefaction process; that is, the combined action of moisture and heat. Since these substitutes are used solely for the extract they yield in the mash tun an estimation of the matter capable of being dissolved by malt extract is of importance.

This extract may be measured by mashing a mixture of equal weight of flaked maize or rice or 1/3 flakes and 2/3 malt under the same

conditions as a black malt (see above) the resulting extract due to the flakes being multiplied by 2 or 3, as the case may be. It is preferable to mash equal quantities of flakes and malt, as any error in analysis is multiplied by 2 instead of 3. J. L. Baker, (*Brewers' Jour.*, 1905, 41, 186) has pointed out that the extract obtained from flakes differs with the diastatic capacity of the malt employed. If deliveries of flakes are controlled by analysis, the same malt should be used by the chemist of the buyer and seller. In this way only is it possible to obtain comparable results. A malt of a diastatic power not exceeding 30° should be used. Briant (*J. Inst. of Brew.*, 1905, 11, 395) suggests mashing the flakes with an extract prepared by digesting a pale malt of a diastatic power of 30°–40° Lintner with three times its weight of cold water for 90 minutes. 20 grm. of the flakes are placed in a beaker, 120 c.c. of water added and the temperature raised to 160° F., carefully stirring during the time. 50 c.c. of the cold water malt extract are run slowly in, the whole mixed and allowed to stand at a temperature of 150° F. for 2 hours. The mash is transferred to a 200 c.c. flask, cooled to 60° F., and made up to bulk, filtered and the sp. gr. taken. This, less the gravity due to the added malt extract (which is treated in a similar manner) represents the gravity due to the flakes. The excess gravity multiplied by the factor 3.32 will give the extract yielded by 336 pounds of the flakes. (The volume occupied by the grains from 20 grm. of flaked maize is, on an average 2.5 c.c., and the factor has been calculated after allowing for this). The method gives satisfactory results

In judging the suitability of flakes for brewing purposes the amount of oil should be noted, as this constituent may impart an unpleasant flavour to the finished beer. The oil may be estimated by extracting 5 grm. of the finely powdered flakes in a continuous extractor with ether for 3 hours. The ether is evaporated off and the residual oil dried in a boiling water-bath for 1 hour, cooled in a desiccator and weighed. Carefully prepared flakes contain about 1% of oil; if more than 2% is present they are not suited for brewing.

Moisture is estimated as in malt. An average figure is 6 to 8 %.

GRITS AND RAW GRAIN.

These are used as a source of extract in some breweries. They are treated in a converter to gelatinise the starch, cooled to a convenient temperature and mashed with malt. Such materials may be analysed

by heating with water preferably under pressure, and treating the starch paste so produced with malt or malt infusion of known extract. Grits should also be examined for oil and moisture.

· MALT EXTRACT.

Malt extract occurs as a light yellow or amber-coloured, thick, viscid liquid, having a faint, pleasant, characteristic odour, a sweet mucilaginous taste and a distinct acid reaction. It is soluble in all proportions in water, the solution being precipitated by strong alcohol. Its diastatic activity is destroyed at temperatures above 65°.

The medicinal value of malt extract depends upon the proportion of total solid nutritive carbohydrates it contains and upon its diastatic action. Many of the extracts on the market contain little or no diastase, the enzyme having been destroyed during evaporation.

The following analyses of commercial preparations were made by A. R. Ling (*Analyst*, 1904, 29, 244):

	I	II	III	IV	V	VI
Sp. gr. 15.5 15.5°.	1395.70 per cent.	1395.12 per cent.	per cent.	per cent.	1408.43 per cent.	1377.82 per cent.
Maltose (apparent)	31.1	30.9	24.8	27.4	34.2	25.2
Dextrose	17.2	18.2	22.0	19.1	12.5	20.0
Dextrin (apparent)	9.8	8.6	10.0	9.8	9.9	6.7
Unfermentable matter (expressed as dextrin)	4.5	3.5	8.9	5.8	......	......
Ash	1.45	1.49	1.58	1.64	1.34	1.64
Water	24.30	24.67	27.36	24.84	24.38	29.52
Diastatic power (Lintner)	30.8	27.2	32.3	25.6	39.2	46.5
Specific rotatory power $[a]_D$	91.8°	90.5°	84.2°	86.8°	94.5°	81.1°

According to W. J. Sykes and C. A. Mitchell (*Analyst.*, 1901, 26, 230), the total solids range between 75 and 82 %; phosphoric acid (P_2O_5), between 0.5 and 1.15, and total nitrogen, between 0.4 and 2.25. The presence of dextrose in authentic samples of malt extract, as pointed out by Ling (*loc. cit.*), is of importance and should be borne in mind when adulteration with "glucose syrup" is being sought for.

The liquid malt extracts largely sold in the United States usually contain but a small amount of carbohydrates, no active diastase, and from 0.5 to 5% of alcohol.

The methods for the estimation of total solids, ash and phosphoric acid are the same as those used in the analysis of malt. It is not possible to estimate the sugars in terms of apparent maltose and dextrin

by the copper method in conjunction with the polarimeter. Ling (*loc. cit.*) determines the dextrose as glucosazone (see under sugar), the maltose being calculated from the reducing power less that due to the amount of glucose found, whilst the dextrin is calculated from the rotatory power after deducting that due to the dextrose and maltose.

Arsenic.—The estimation of this impurity is somewhat out of place in a volume devoted to "organic analysis," but since the alarm occasioned by the arsenical contamination of beer in 1900 to 1901, the testing of brewing materials and beers for arsenic has become a matter of routine. Although excellent methods have been devised for this, the Marsh-Berzelius process, with recent improvements, is, in the opinion of the writer, the simplest.[1]

The reports, minutes of evidence and appendices on the Royal Commission of Arsenical Poisoning, 1902, may be regarded as a text-book on this subject. The standard electrolytic method is described at length in Appendix 21, page 208 of the report.

PREPARATION OF MATERIALS.

Zinc.—Since arsenic is frequently present in zinc it is necessary to ascertain if the latter is free from this impurity and also if it is sufficiently sensitive, or in other words, if it produces a normal arsenic deposit from a solution containing a known amount of arsenious oxide. Before use the zinc must be granulated. The outer surface of the ingot is cleaned by scraping, then treated with arsenic-free hydrochloric acid and well washed with water. The zinc is melted in a porcelain crucible and when just molten it is poured from a height of about 4 feet into cold water. Chapman (*Analyst* 1907, **32,** 247.) has proposed the addition of a cadmium salt to increase the sensitiveness of the zinc. Many dealers now supply granulated zinc sufficiently pure to be used in the estimation.

Hydrochloric Acid.—This acid unless specially purified frequently contains sufficient arsenic to render its application for the test useless. The method devised by Thorne (*Proc. Chem. Soc.*, 1902, **18,** 118) works well in practice. Ordinary strong hydrochloric is diluted with water and placed in a large retort. Through the stoppered opening is introduced a glass rod carrying on the end a piece of very fine copper

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[1] This method is similar to that recommended by a joint committee of the *Society of Public Analysts* and the *Society of Chemical Industry* (*J. Soc. Chem. Ind.*, 1902, 21, 94).

gauze. The contents of the retort are gently boiled for an hour, the glass rod and copper gauze removed, a small piece of fresh gauze added and the acid distilled. The first 100 c.c. of the distillate should be rejected. The purified acid used in the test should have a sp. gr. 1.1.

Apparatus.—The flask is fitted with a ground-glass stopper through which passes the stem of a funnel furnished with a stop-cock. The stopper also carries the exit tube on which is a bulb and which is bent twice at right angles and connected with the tube containing calcium chloride and a plug of lead acetate paper. The hard glass tube on which the arsenic is to be deposited is made of Jena tubing (external diameter 5 mm.), drawn-out portion having a diameter of 2 mm.,

FIG. 53.

with the end turned up at right angles. A piece of platinum gauze should be wrapped round the tube at the point at which it is to be heated by the Bunsen flame.

Preparation of the Substances to be Tested.—Unground Malt.— 40 grm. of the malt are treated at a temperature of 50° for twenty minutes with 40 c.c. of a mixture of hydrochloric acid, sp. gr. 1.1 and 60 c.c. of water. 25 c.c. of the supernatant liquid are used for the test.

Malt Substitutes.—A 20 % solution of glucose, invert sugar, or caramel is made and acidified with hydrochloric acid.

Worts, Commercial Malt Extracts, and Caramels.—It is usually preferable to destroy the organic matter in these materials as the pres-

ence of the dextrinous and protein matters retards the formation of hydrogen arsenide. The destruction of these substances may be effected with lime and magnesia or by treatment with fuming nitric acid (compare *Rep. Royal Commission on Arsenical Poisoning, Appendix* 21, page 213).

Hops and Hop Substitutes.—A weighed quantity is treated with dilute hydrochloric acid and an aliquot portion of the extract used for the test, or the organic matter may be destroyed by incinerating with lime and magnesia and the ash dissolved in hydrochloric acid.

Yeast and yeast foods are treated in the same way as worts.

Beers.—Generally beers may be introduced directly into the apparatus. Some beers of high gravity behave like worts, and in such cases the organic matter should be destroyed.

Method of Working.—10 grm. of granulated zinc are placed in the flask A and covered with a little dilute arsenic-free hydrochloric acid. After the action has proceeded for two or three minutes, it is well washed and the necessary connections made. 10 c.c. of the pure hydrochloric acid (sp. gr. 1.1) are gradually added. At the end of 10 minutes the apparatus will be practically free from air, and the issuing hydrogen may be lighted. At the same time the burner is also lighted, and the heating of the hard glass tube so regulated that the piece of platinum gauze is maintained at a red heat. Then during 20 minutes a further quantity of 10 c.c. of hydrochloric acid is added. The hydrogen flame should be from 2 to 3 mm. in height and the acid is to be added throughout the experiment so as to secure this. During the 20 minutes heating of the tube a deposit of arsenic, best seen by holding a white card beneath the tube, will be formed if the zinc or acid is not arsenic-free. In such a case the experiment must be discontinued, the flask washed out and fresh materials employed.

When the materials are thus proved to be free from arsenic, the solution to be tested is gradually run in, so that its addition to the generating flask is spread over a period not exceeding 15 minutes, and the hydrogen flame is maintained at a height of 2 to 3 mm. When the whole of the solution has been added, the generation of the hydrogen is continued for another 15 minutes at least, by the addition, as required, of more hydrochloric acid. For that purpose from 10 to 15 c.c. are needed.

Preparation of the Standard Deposits.—The standard deposits with which the arsenic deposits from tested substances are to be compared must be prepared by the use of a specimen of each kind of sub-

stance containing known amounts of arsenous oxide. The quantity of substance taken and the manner of preparing the solution or extract must be the same as described under the test for that substance. Every care should be taken that the period of time over which the solution is added, the size of the hydrogen flame, the mode and duration of heating of the glass tube, and the amount of acid used, should be the same in the preparation of the series of the standard deposits as in the carrying out of the actual test. The mirrors as soon as deposited should be sealed at both ends in an atmosphere of hydrogen and kept in the dark. According to the experience of the writer the standard mirrors remain practically permanent for three months.

The standard arsenic solution is prepared by dissolving 0.1 grm. of pure arsenous oxide in a small quantity of pure strong hydrochloric acid. The liquid should not be heated. When the solution is complete it is diluted to 1000 c.c. with distilled water. 1 c.c. of this solution contains 0.1 mg. of arsenous oxide.

MALT LIQUORS.

Beer, Ale.

Beer may be described as a fermented liquor brewed from malt or from a mixture of malt and malt substitutes and having a bitter flavour communicated by hops or by other wholesome bitter. In the Middle Ages ale was a fermented infusion of malt and water flavoured with a small quantity of some bitter principle, such as oak bark. Beer, on the other hand, was made from malt, water and hops. The distinction between ale and beer lasted for a considerable time. Hops gradually came into general use, but the word ale was retained whether the liquor designated by it was hopped or not. The word "beer" now includes all malt liquors, whilst ale includes all but black or brown beers.

Under the present law of England, the malt of typical beer may be replaced by any saccharine or amylaceous substance, and as the duty is levied on the quantity of soluble carbohydrates made into beer, as determined by the sp. gr. of the infusion, the exact nature of the fermentable matter employed is a matter of indifference to the Excise. Similarly, the employment of hops is not insisted on by the Excise, and any wholesome bitter (e. g., quassia and gentian) can be employed. The

substitution is not an infringement of the Sale of Food and Drugs Act, which could, however, be enforced in the case of a distinctly unwholesome bitter being used. It may, however, be pointed out that hop substitutes are only employed to a very slight extent in breweries; according to the Excise returns of last year 63,936,409 pounds of hops were used for brewing and only 29,502 pounds of hop substitutes.

The chemical composition of beer and other malt liquors is very complex, the main constituents may be conveniently arranged in the following three classes:

a. The volatile constituents; including alcohol, water, acetic acid, carbonic acid and some other acids.

b. The fixed organic matters, forming the organic constituents of the "extract"; including sugars, dextrins and dextrinoid bodies, glycerol, lactic and succinic acids, proteins, and organic extractive matters from hops, etc.

c. The mineral constituents or ash; consisting chiefly of potassium, calcium, and magnesium phosphates.

Beer differs from wine in its smaller content of alcohol, and the greater proportion of dextrin and other extractive matters present; also in the absence of acid tartrates, which are characteristic of wine as malic acid is of cider and lactic acid of beer. The acidity of beer is frequently ascribed to acetic acid, but, except in sour ales, it is chiefly due to lactic acid, to other organic acids produced by fermentation, and acid phosphates.

The composition of malt liquors differs widely according to the nature and proportion of the materials used and the manner in which the fermentation has been conducted. Broadly speaking, two distinct methods of brewing are pursued, namely, the German and the English. German beers are fermented at a low temperature, under which condition the yeast remains at the bottom of the liquid, and the process is said to be one of "bottom-fermentation." The yeast is a different variety from that of English breweries. Beer brewed on this system contains less alcohol and more dextrin, sugar, and nitrogenous matter than English beer, and hence is liable to undergo secondary fermentation unless kept at a very low temperature or else sterilised and preserved in bottles. The German beer also contains less hops than English beer. In the English system of brewing, the operation is one of "top-fermentation," and as a rule the product is richer in alcohol and contains less extractive matter than German beer.

Generally, bitter ales have a low attenuation, high percentage of alcohol and much hop extract; mild ales higher attenuations, less alcohol and less hop extract; porter about the same attenuation as mild ale, but less hops. Stouts usually have a high attenuation and low alcohol content; they are hopped in proportion to their gravity. Export ales and stouts have low attenuations and high content of alcohol and are heavily hopped. A lengthy list of the different beers of the world and their analyses may be found in Wahl and Henius' *Handy Book* of *Brewing and Malting*. A full analysis of a beer is useful for technical control purposes, but given two beers brewed at the same gravity it is not possible, in the opinion of the writer, to adduce figures to show that one is of superior quality to the other. An analysis will give some information as to how a beer was brewed and it is also possible by the "Forcing Test," which will be described later, to form an idea as to the stability of a beer and how it will behave in the trade.

Original Gravity of Beer-worts.—As the duty on beer is calculated from the strength of the wort as indicated by its sp. gr., it becomes necessary to allow a rebate or drawback when the beer is exported. If the wort could always be examined in an unfermented state, it would merely be necessary to ascertain its density and gauge its measure to obtain the data for calculating the allowance to be made. But by the process of fermentation the sp. gr. of the wort is diminished to an extent dependent on the amount of alcohol formed. The weight of alcohol produced being approximately 50 per cent. of the saccharine matter destroyed by the fermentation, it is evident that a determination of the alcohol in the fermented liquid would give the means of ascertaining the quantity of sugar destroyed, and hence of making the necessary correction for the reduction in the density of the wort (technically called its "attenuation") caused by the fermentation.

The practical details of the methods of estimating the original gravities of beer worts have been investigated by Graham, Hofmann, and Redwood, and their results show that that the information can be obtained in the following manner:

Distillation Method.—The carbon dioxide is first removed from the sample of beer to be examined by "tossing," that is pouring from one beaker to another, or by filtering through paper or a plug of glass wool.

100 c.c. of the beer at a temperature of 60° F. are introduced into the distilling flask (Fig. 54) of the original gravity apparatus by a

pipette, 40 c.c. of water added and distillation is continued until about 80 c.c. have passed over. The distillate is made up to 100 c.c. at 60° F. and the sp. gr. determined. The residue in the distilling flask is cooled and transferred, together with washings, into a 100 c.c.

FIG. 54.

flask made up to volume with water at 60° F. and the sp. gr. ascertained. The sp. gr. of the distillate represents the fermented matter as a mixture of alcohol and water, and that of the residue the unfermented matter in the original wort. To find the amount of fermented matter

the sp. gr. of the alcohol distillate is subtracted from 1,000 and the difference is the "spirit indication number" (see Table I). From this table the number of degrees of sp. gr. lost during fermentation which correspond to the "spirit indication" may be found. This number plus the sp. gr. of the unfermented matter represents the original gravity of the wort.

TABLE I.

SPIRIT INDICATION TABLE SHOWING DEGREES OF GRAVITY LOST IN MALT WORT DURING FERMENTATION.

Degrees of Spirit Indication.	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0	..	.3	.6	.9	1.2	1.5	1.8	2.1	2.4	2.7
1	3.0	3.3	3.7	4.1	4.4	4.8	5.1	5.5	5.9	6.2
2	6.6	7.0	7.4	7.8	8.2	8.6	9.0	9.4	9.8	10.2
3	10.7	11.1	11.5	12.0	12.4	12.9	13.3	13.8	14.2	14.7
4	15.1	15.5	16.0	16.4	16.8	17.3	17.7	18.2	18.6	19.1
5	19.5	19.9	20.4	20.9	21.3	21.8	22.2	22.7	23.1	23.6
6	24.1	24.6	25.0	25.5	26.0	26.4	26.9	27.4	27.8	28.3
7	28.8	29.2	29.7	30.2	30.7	31.2	31.7	32.2	32.7	33.2
8	33.7	34.3	34.8	35.4	35.9	36.5	37.0	37.5	38.0	38.6
9	39.1	39.7	40.2	40.7	41.2	41.7	42.2	42.7	43.2	43.7
10	44.2	44.7	45.1	45.6	46.0	46.5	47.0	47.5	48.0	48.5
11	49.0	49.6	50.1	50.6	51.2	51.7	52.2	52.7	53.3	53.8
12	54.3	54.9	55.4	55.9	56.4	56.9	57.4	57.9	58.4	58.9
13	59.4	60.0	60.5	61.1	61.6	62.2	62.7	63.3	63.8	64.3
14	64.8	65.4	65.9	66.5	67.1	67.6	68.2	68.7	69.3	69.9
15	70.5	71.1	71.7	72.3	72.9	73.5	74.1	74.7	75.3	75.9

The experimental data on which the table was constructed included the formation of 0.1 % acidity calculated as acetic acid, and no correction is necessary in the case of beers containing about this proportion. Any excess of acidity over 0.1 % is supposed to be formed at the expense of the alcohol in the beer, and unless this acidity is allowed for the original gravity will be low. The amount of acid present in the beer is estimated by N/10 ammonium hydroxide using litmus paper as an indicator. From the result 0.1% is subtracted and the difference referred to Table II which indicates the correction due to the excess of acid formed. This number is then added to the spirit indication figure.

MALT AND MALT LIQUORS.

TABLE II.

TABLE FOR ASCERTAINING THE CORRECTION FOR ACID.

Excess per cent. of acetic acid in beer	Corresponding Degrees of Spirit Indication.									
	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
.0	..	.02	.04	.06	.07	.08	.09	.11	.12	.13
.1	.14	.15	.17	.18	.19	.21	.22	.23	.24	.26
.2	.27	.28	.29	.31	.32	.33	.34	.35	.37	.38
.3	.39	.40	.42	.43	.44	.46	.47	.48	.49	.51
.4	.52	.53	.55	.56	.57	.592	.60	.61	.62	.76
.5	.65	.66	.67	.69	.70	.71	.72	.73	.75	.64
.6	.77	.78	.80	.81	.82	.84	.85	.86	.87	.89
.7	.90	.91	.93	.94	.95	.97	.98	.99	1.00	1.02
.8	1.03	1.04	1.05	1.07	1.08	1.09	1.10	1.11	1.13	1.14
.9	1.15	1.16	1.18	1.19	1.21	1.22	1.23	1.25	1.26	1.28
1.0	1.29	1.31	1.33	1.35	1.36	1.37	1.38	1.40	1.41	1.42

When a beer has become very acid with acetic acid it is necessary to make allowance in respect of it; firstly, because some of the alcohol formed has been lost by being converted into acetic acid and, secondly, because the acetic acid will distil over with the alcohol and raise the sp. gr. of the distillate and consequently reduce the apparent spirit indication and also the original gravity. In such cases, Moritz and Morris (*Text-Book of the Science of Brewing*, p. 503) advise that a second distillation be performed in presence of sufficient alkali to neutralise the acid and so prevent the distillation of the acetic acid. To make a correction for the loss of alcohol by conversion into acid the acetic acid is estimated as follows: 100 c.c. of the beer are taken and titrated with ammonia of 998.6 sp. gr., red litmus paper being used as an indicator. Each c.c. of ammonia used represents 0.1 of acetic acid. This will give the *total acid* of the beer calculated as acetic acid. 100 c.c. of beer are evaporated to dryness on a water bath, the residue redissolved in water and titrated as before. This gives the *fixed acid* calculated as acetic acid. The difference between the two gives the volatile acid, or real acetic acid. The above percentage of acid is then referred to the table above for corresponding loss of spirit indication, and this spirit indication is then added to that obtained by distillation. The method of calculation will be seen from the following example:

Sp. gr. of *water* at 60° F.,	1000.0
Sp. gr. of *distillate* at 60° F.,	989.0
Difference = "*spirit indication*,"	11.0
Allowance for alcohol corresponding to 0.2 % *excess* of acid,	.27
Corrected spirit indication,	11.27
Equal, by table, to "*gravity lost*,"	50.4
To which add sp. gr. of *extract*,	1041.3
Original gravity of wort,	1091.7

The table already given (page 153) is the only one legalised for the determination of original gravities, and is used by the Excise, without correction, whether the wort be derived wholly or partly from starch- or cane-sugar, or simply from malt. This practice gives the brewer the advantage of any error.

With worts containing yeast which have just started fermentation the results are fairly accurate, but the original gravities of finished beers are usually about 2° too low. It cannot be expected that a partially fermented and fully fermented mixed malt and sugar wort will give the same original gravity, as the ratio of residual matters left after fermentation to the alcohol is different with malt worts as compared with sugar worts.

Evaporation Method.—In employing this process, the sp. gr. of the original beer is first carefully ascertained, taking care to agitate the liquid well to eliminate as much carbonic acid as possible. The "extract gravity" is next determined. For this purpose there is no occasion to boil the sample in a closed vessel, as it is not required to collect the volatilised spirit. It is simply necessary to evaporate sufficiently to insure the entire expulsion of the alcohol, and then allow the liquid to cool, and make it up exactly to the original bulk of the beer taken. The sp. gr. is then observed, and the corresponding "spirit indication" ascertained by subtracting the sp. gr. of the original beer from that of the "extract." The necessary allowance, if any, for excess of acid above 0.1% must next be made as in the distillation method, and from the corrected spirit indication the corre-, sponding number of degrees of gravity lost is ascertained by reference to the table already given. The result thus obtained is not in strict accordance with that by the distillation method, and requires to be corrected by an addition of 1/40 to the "degrees of gravity lost" as ascertained by the table. Thus, if the corrected spirit indication be 9.4,

corresponding to 41.2 degrees of gravity lost, the last figure requires a correction of $\frac{41.2}{40} = 1.03$, which, added to 41.2 raises it to the corrected number, 42.03 degrees. The following example illustrates the whole mode of calculation:

Sp. gr. of "*extract*,"	1044.7
Sp. gr. of original *beer*,	1035.2
Difference = "*spirit indication*,"	9.5
Allowance for excess of acidity,	0.1
Corrected spirit indication,	9.6
Corresponding "*gravity lost*" (by table),	42.2
Correction of 1/40 of above number,	1.055
Corrected gravity lost,	43.25
Sp. gr. of *extract*,	1044.7
Original gravity of wort,	1087.95

The results by the evaporation process are not generally so reliable or so constant on repetition as those by the distillation method, but they are obtained with great facility, the only additional operation necessary being the determination of the density of the original beer, and hence the calculation should never be omitted, as it furnishes a valuable check on the distillation process.

The Optical Method of Determining Alcohol and Extract in Beer.—At the request of the Norwegian Government, H. Tornöe undertook the task of devising a rapid and simple method of beer analysis for revenue purposes. He elaborated a process whereby the amount of alcohol and extract in a beer can be ascertained in about 10 minutes. The measurements involved are the sp. gr. of the beer at 63.5° F. and the index of refraction of the beer for sodium light at the same temperature. The principles involved and the use of the instrument have been fully described by Ling and Pope (*J. Fed. Inst. of Brewing*, 1901, **7**, 170). The agreement between the original gravities as determined by Tornöe's method and the distillation method is satisfactory. (Compare also Race, *J. Soc. Chem. Ind.*, 1908, **27**, 544.)

Estimation of Alcohol.—The amount of alcohol in a fermented wort or beer is obtained by referring the sp. gr. of the alcoholic distillate such as is obtained in the original gravity determination (see page 151) to the alcohol tables which show the weight of alcohol corresponding to a given sp. gr. of aqueous alcohol; from the weight found that of alcohol in 100 c.c. of the original beer is calculated.

Estimation of Extract.—The proportion of extract or matter remaining in a beer may be deduced from the sp. gr. of the de-alcoholised liquid obtained by evaporating the sample to one-third and diluting again to its original bulk. The sp. gr. of the extract is then observed, and the excess above 1,000 divided by 4, the quotient being the number of grms. of dry extract contained in 100 c.c. of the beer. Or the residue left in the distillation flask in the original gravity determination may be used for this purpose. The "apparent maltose" and "apparent dextrin" may be determined in the extract in the manner described on page 141

The Amount of Unfermentable Matter in a Beer.—This determination is of value in forming an opinion as to the probable course of the so-called "secondary" fermentation, and the resulting condition of the beer. When systematically made it also affords information of the suitability of the pitching yeast used in a brewery.

100 or 200 c.c. of the beer are evaporated until the alcohol is removed, made up to the original volume and fermented with 1 or 2 grms. of pressed yeast for 48 hours. The solution is then boiled to expel alcohol, made up to the original volume with water and a small quantity of alumina cream and filtered. The grm. of maltose per 100 c.c. are then determined from the reducing power (see page 136) and the difference in the amount of maltose before and after fermentation represents the amount of fermentable matter remaining in the beer. This difference, although conveniently expressed as maltose, includes easily fermentable low-type malto-dextrins, etc.

Total Nitrogen.—This may be estimated by the Kjeldahl process. 25 c.c. of the beer are evaporated to a small bulk in the flask in which the decomposition with sulphuric acid is carried out, a few drops of dilute sulphuric acid added, and the whole taken nearly to dryness and then heated with 20 c.c. of strong sulphuric acid and potassium sulphate in the usual way. The nitrogen multiplied by the factor 6.25 gives the total protein percentage.

Estimation of Carbon Dioxide in Beer.—Since carbon dioxide may be regarded as the natural preservative in beer its estimation is of some importance. English beers in cask contain between 0.25 and 0.35 per cent. carbon dioxide; if there is less than 0.2 % the beer tastes flat. Windisch (*Das chemische Laboratorium des Brauers*, page 326) describes the following process (Fig. 55) which gives satisfactory results:

A known weight of beer, about 300 grm., is placed in the flask "A."

The screw clip "a" which is closed in the first part of the estimation controls the entry of air from the soda-lime tower "T"; "b" and "b" connect with the condenser "B." When the contents of the flask are boiled most of the alcohol and steam is condensed by means of "B"; the calcium chloride tube "C" retains any aqueous vapour which may escape. The bulbs "D" contain strong sulphuric acid and "E" strong potassium hydroxide for absorbing the carbon dioxide. When gas ceases to be absorbed in "E" "g" and "h" are connected and air is

FIG. 55.

drawn through the apparatus by means of the aspirator "H," the screw clip "a" being opened slightly at the same time. "F" is packed with small pieces of potassium hydroxide and "G" with calcium chloride. About 12.50 c.c. of air aspirated through the apparatus will suffice to remove all traces of carbon dioxide from the beer. The flame is then taken from under the flask "A," "E" is disconnected and in due course weighed. The difference in weight before and after the experiment is the amount of carbon dioxide absorbed.

If it is desired to estimate the amount of carbon dioxide in a bottled beer the cork is pierced with a pointed hollow tube carrying a tap. The exit from the tap is connected with "C" by the rubber joint "m"

and the gas allowed to slowly escape. The bottle, still attached to "m," is then placed in a water-bath which is raised to b. p. When gas ceases to be given off the bottle of beer is removed, the tube "C" connected with the condenser and the beer when cold placed in "A." The contents of the flask "A" are boiled and air aspirated through the apparatus as before.

A. O. A. C. PROCESS (*Bulletin* 107, Bur. of Chem., U. S. Dept. of Agric.):

Bottled Beers.—Pierce the cork with a champagne tap. (Crampton found it advantageous to re-grind the cocks and ream off the

FIG. 56.—Crampton and Trescot; Bulletin 107, Bur. of Chem. U. S. Dept. Agric.

thread.) The bottle thus tapped is connected with the absorption apparatus shown in Fig. 56, devised by Crampton and Trescot. The can containing the bottle holds a convenient amount of cold water. The tap is opened so as to allow the gas to escape slowly, and when the flow ceases, the water is heated slowly to about 80°, shaking the bottle from time to time during about 30 minutes while this temperature is maintained. The bottle is then disconnected and air under the usual precautions drawn through the apparatus. The increase in weight of the absorption tube gives the amount of carbon dioxide. The contents of the bottle are either weighed or measured to give the necessary data for calculation. Bottles carrying patent stoppers can sometimes be adapted to this method by substituting quickly a rubber stopper fitted with a suitable stopcock tube. When this cannot be done, the method given in the next paragraph must be employed.

Bulk Beers.—A round-bottom flask, about 700 c.c. capacity, is provided with a rubber stopper carrying two stopcock tubes, each bent at right angles, one tube passing to the bottom of the flask, the other terminating just below the stopper. A partial vacuum is produced in the flask which is then weighed. The end of one of the stopcock tubes is then dipped below the surface of the sample and about 300 c.c. allowed to enter the flask, which is then weighed, and the procedure for bottled samples followed. It is recommended as a better manipulation to attach to one of the stopcock tubes, by means of a rubber tube, a champagne tap that has been screwed into the cask. Somewhat better results may be obtained by placing a reflux condenser between the flask and absorption apparatus and heating the flask until the contents boil.

The Mineral Constituents of Beers.—The total ash of a beer may be estimated by evaporating 50 c.c. of the sample to dryness in a large platinum crucible, and cautiously igniting at a low red heat in a muffle. An estimation of certain of the ash constituents is sometimes useful in determining if a beer is correctly described as the product of a certain brewery. For example, a brand of beer may be brewed to contain certain proportions of gypsum or chlorides. An estimation of these constituents would afford material evidence in detecting a case of fraudulent substitution.

In instances where the addition of large quantities of sodium chloride is suspected a quantity of the beer (50 c.c.) should be evaporated to dryness in the presence of sodium carbonate and ashed in a muffle at as low a heat as possible. The total chlorine in the ash is estimated gravimetrically. Race (*J. Soc. Chem. Ind.*, 1908, **27**, 548) recommends evaporating 50 c.c. of the beer with 0.5 grm. of barium carbonate and subsequently igniting to a black ash. The ash is extracted with hot water, filtered, and titrated with silver nitrate in the usual manner.

Another volume of 50 c.c. of the beer is evaporated to dryness, moistened with sulphuric acid and ashed. The potassium is estimated as chloroplatinate and the sodium by difference. From these data and the chlorine the amount of sodium chloride present in the beer may be calculated. Beer may, under English regulations, contain 50 grains of sodium chloride per imperial gallon, (41.5 grains per U. S. gallon) this amount being derived from the treatment of the mashing liquor and from the malt and malt substitutes used.

Sulphates.—50 c.c. of the beer are evaporated to dryness in the presence of a small quantity of sodium hydroxide, the mass ashed and the sulphates estimated in the usual manner. If the beer is burnt by itself loss of sulphuric anhydride occurs owing to the interaction between acid phosphates and the sulphates.

The question of whether a beer is made from an all-malt grist or part malt and part substitute is often asked. It may be asserted that with a few exceptions nearly all brewers use substitutes. In the United Kingdom the malt and hops comprise 83% of the solid materials used in brewing, the remaining 17% including unmalted corn, malt substitutes, and sugars. The nature and proportion of nitrogenous matter has been suggested as a means of detecting substitutes, but when the varying composition of malt is borne in mind it will be realised that no safe conclusion can be drawn from such data. The fact that the amount of phosphoric acid is higher in an all-malt beer than in one brewed with substitutes has been proposed as a means of detecting substitutes. The amount of phosphates, however, differs in malt, also the quantity taken up in the development of the yeast is not constant. Hence the evidence afforded by this estimation is only diagnostic and and not conclusive.

Detection of Bitter Substances in Beer.—Very elaborate processes have been devised by Dragendorff, Wittstein and others for detecting the presence of substances which might possibly be used for imparting a bitter taste to beer, but it will be sufficient to describe here the method of searching for the more commonly used "hopsurrogates," and certain objectionable substances the occasional employment of which is suspected.

A. C. Chapman (*Analyst*, 1900, 25, 35) has devised a method for distinguishing between hops and quassia, which is based upon the production of valeric acid when the ether extract of hops is oxidised with an alkaline solution of potassium permanganate. 500 c.c. of the beer are evaporated on the water-bath with the addition towards the end of the operation of some ignited sand, the mass being constantly stirred to prevent it from adhering to the surface of the dish. The residue is dried in an air oven, finely powdered and extracted in a bottle with ether. The ether is removed from the extract and the residual matter oxidised by the careful addition of an alkaline solution of potassium permanganate containing 40 grm. of permanganate and 10 grm. of potassium hydroxide in 1000 c.c. This solution should be added

in small quantities at a time, the flask being vigorously shaken and if necessary warmed. When the permanganate ceases to be readily reduced, a few drops of a hot solution of oxalic acid are added to complete the reduction, and the colourless liquid filtered from the manganese oxides into a glass dish, in which it is evaporated to dryness. The dry residue is then acidified with dilute sulphuric acid, when the odour of valeric acid in the case of the hop-bittered liquid becomes at once apparent, being rather accentuated by the carbon dioxide liberated at the same time from the potassium carbonate formed during the oxidation. The smell observed is not that of pure valeric acid, but of valeric acid plus some other odorous compound, which serves to render it more characteristic. In the case of the quassia the liberated acid is chiefly acetic. Old hops respond as readily to this test as new hops. Camomile extract behaves in a similar manner to hops, but chiretta yields no valeric. acid.

OUTLINE PROCESS FOR THE DETECTION OF BITTER PRINCIPLES IN BEER.

1000 c.c. of beer is evaporated to half its bulk and precipitated boiling with neutral lead acetate, the liquid boiled for fifteen minutes and filtered hot. If any precipitate separates cooling, the liquid is again filtered.

Precipitate contains *hop*-bitter, *caramel* - bitter. *ephelic acid* (from chiretta), phosphates, albumenous matters, etc., etc.

Filtrate. The lead is removed by hydrogen sulphide and the filtered liquid concentrated to about 150 c.c. and tasted. If any bitter taste is perceived, the liquid is then slightly acidulated with dilute sulphuric acid, and shaken repeatedly with chloroform.

Chloroform layer, on evaporation, leaves a bitter extract in the case of *gentian, calumba, quassia,* and *old hops* (only slightly or doubtfully bitter in the case of chiretta). The residue is dissolved in a little alcohol, hot water added, and the hot solution treated with ammoniacal basic lead acetate and filtered.

Aqueous liquid is shaken with ether.

Ethereal layer leaves a bitter residue in the case of *chiretta, gentian,* or *calumba.* It is dissolved in a little alcohol, hot water added, and the hot solution treated with ammoniacal basic lead acetate and filtered.

Aqueous liquid, if still bitter is rendered alkaline and shaken with ether - chloroform. A bitter extract may be due to *berberine* (calumba) or *strychnine.*

Precipitate contains the bitters of *old hops, gentian,* and *caramel.* It is suspended in water, decomposed by hydrogen sulphide, and the solution agitated with chloroform.

Filtrate is boiled to remove ammonia, and treated with a slight excess of sulphuric acid filtered and tasted. If bitter, it is agitated with chloroform, and the residue examined for *calumba* and *quassia.*

Precipitate is treated with water and decomposed by hydrogen sulphide. The filtered liquid is *bitter* in presence of *gentian.*

Filtrate is treated with a slight excess of dilute sulphuric acid, filtered and tasted. A bitter taste indicates *calumba* or *chiretta,* which may be re-extracted with ether and further examined.

The aqueous liquid, separated from the ether-chloroform, may contain *caramel - bitter* or *choline* (somewhat bitter).

Chloroform Layer is examined by special tests for *gentian* and *old hop*-bitter.

Aqueous liquid contains traces of *caramel*-*bitter.*

This method is applicable to the examination of hop-bitter preparations (of a medicinal character), hop extracts, and similar products. It furnishes additional evidence in the case of fermented beverages which have been examined according to the systematic schemes in vogue, and which have yielded results of an uncertain nature.

Preservatives in Beer.—The most commonly occuring preservative is sulphurous acid, usually as a sulphite or, rarely, in the free state. Salicylic acid is also used, often in association with a sulphite. Fluorides are occasionally found in continental beers.

Salicylic Acid.—The following process devised by F. T. Harry and W. R. Mummery (*Analyst*, 1905, **30**, 124-127) gives satisfactory results:

100 c.c. of the beer are placed in a graduated 200 c.c. flask made alkaline with 5 c.c. normal sodium hydroxide and the alcohol driven off at a temperature just below the b. p. After cooling 5 c.c. of normal hydrochloric acid are added and 20 c.c. of basic lead acetate solution; the mixture is then made alkaline with about 20 c.c. of $N/1$ sodium hydroxide and made up to 200 c.c. At this stage the solution may be raised to boiling and allowed to cool before filtering, but this may be omitted if thought advisable. 100 c.c. of the filtrate are acidified with hydrochloric acid, a precipitate of lead chloride being thrown down and filtered off. The filtrate is extracted with ether three times, the ether distilled off and the salicylic acid dissolved in a small quantity of dilute alcohol and made up to 100 c.c. The salicylic acid is estimated colorimetrically in ordinary 50 c.c. Nessler tubes with very weak ferric chloride solution which should be made up freshly when required. The standard salicylic solution is 0.01 % strength. The tendency which beers have to emulsify when shaken with ether is obviated by this process.

Sulphites.—5 c.c. of phosphoric acid are added to 300 c.c. of beer, the mixture distilled and $N/100$ iodine solution run in until there is a permanent yellow color. The excess of iodine is determined by $N/100$ sodium thiosulphate solution in the usual manner. One c.c. $N/100$ iodine is equivalent to 0.00032 grm. SO_2.

Fluorides.—100 c.c. of the beer are made slightly alkaline with ammonium carbonate, boiled and 2 or 3 c.c. of 10% calcium chloride solution added; again boiled for five minutes, the precipitate filtered, washed and dried. The dried precipitate is detached from the paper, placed in a platinum crucible and ignited, then powdered with a small pestle, moistened with 2 or 3 drops of water and 1 c.c. of strong sulphuric acid. The crucible is covered with a watch-glass, the surface of which

is waxed and marked with a style. The crucible and its contents are gently warmed on the water-bath, the wax on the cover-glass removed and any etching on the glass surface noted. To prevent the wax melting during the process, the watch-glass is covered with a larger glass on which pieces of ice are placed.

Saccharin.—The addition of saccharin is forbidden in most countries. Allen (*Analyst*, 1888, **13,** 105) devised the following method for its detection:

The beer is concentrated to 1/3 its bulk, and if not acid, is rendered so by the addition of a little pure phosphoric acid. The liquid is then shaken with ether, the ether decanted and evaporated, and the residue burned off after being mixed with sodium carbonate and a little sodium nitrate. The sulphur in the saccharine is thus converted into sulphate, and can be estimated in the usual way. The weight of barium sulphate multiplied by 0.785 gives the weight of saccharin. Of course, all the reagents must be free from sulphates.

The Stability of Finished Beers.—Useful information as to the keeping qualities of beers and their suitability for certain purposes, such as bottling, exporting to hot countries, etc., may be obtained from the "forcing test." (For a full description of this test see Matthews and Lott, *The Microscope in the Brewery*, 2d ed., page 128.) The samples of beer are placed in carefully cleaned conical flasks fitted with rubber stoppers carrying tubes which dip down into a receptacle containing mercury. The flasks are placed on the outside of a large metal water-bath, maintained at a temperature of 80° to 85° F., or better in an incubator, for certain specified periods which are regulated by the "trade expectations" of the beers under examination. The apparent gravity, acidity, flavour, odour and condition are recorded before and after the test and the sediment which is formed carefully examined for wild yeasts and bacteria.

Beers for export purposes and stock ales and stouts should be perfectly sound and the sediment free from bacteria after 4 weeks' duration of the test. India pale ales should stand three weeks; light bottling ales and stouts a fortnight; and running beers, such as mild ale and porter, a week. The loss in gravity during the forcing test is an indication as to the rapidity with which a beer will get into condition in bottle. It is not possible to discuss the test fully in the present article, but if intelligently used it affords information of considerable diagnostic value to the brewer.

WINES AND POTABLE SPIRITS.

By G. C. JONES, F. I. C., A. C. S. I.

WINES.

Many distinguished chemists have devoted attention to the analysis of wines and new methods or modifications of old ones are proposed annually. Since, however, few of the estimations are absolute, new methods, even if good, are very cautiously received by continental chemists, who hold that it is more important to obtain numbers strictly comparable with those previously accumulated than to increase slightly the accuracy of a single determination. In these circumstances, the reader has a right to expect in a work of this kind a description of official methods of analysis, and with this in mind the writer has tried to steer a middle course between rival official methods, and, where the estimation is one which may be seriously influenced by departure from standard conditions, to point out difference in the official methods of different countries. Frequent reference is made in this section to *Bulletin* No. 59, United States Department of Agriculture (*Division of Chemistry*) which will for the sake of brevity be cited by the author's name (Bigelow). The methods described by Bigelow differ very little from the German methods, which may be found described in great detail in K. Windisch's *Chemische Untersuchung des Weines* (Berlin, 1896). A German imperial decree of 1896 was very fully abstracted in *J. Soc. Chem. Ind.*, 1898, **17**, 277, and will be found to contain an amount of detail concerning analytical methods which is scarcely justifiable in this work.

The following determinations are usually made:

Specific gravity.
Alcohol.
Glycerol.
Extract.
Ash.
Acidity (volatile and fixed).
Sugar.
Potassium sulphate.
Sulphurous acid.

In addition to these it is sometimes necessary to estimate tannin and to look for saccharin, salicylic acid and other preservatives. It is convenient to return all results as grm. per 100 c.c. of wine. The determination of the sp. gr. and estimation of alcohol do not need description here.

Glycerol.—The German official methods are as follows:

a. In wines containing less than 2 grm. of sugar per 100 c.c., 100 c.c. are evaporated to 10 c.c. in porcelain on the water-bath, and the residue mixed with 1 grm. of quartz sand and, for each grm. of extract present 1.5 to 2 c.c. of milk of lime (40% calcium hydroxide). Evaporation is continued almost to dryness and then 5 c.c. of 96% alcohol added. The matter which adheres to the sides of the dish is loosened with a spatula and reduced by a small pestle to a thin paste, further small quantities of 96% alcohol being added as required. The mixture is heated on the water-bath, with constant stirring, until it begins to boil, when the liquid portion is decanted into a 100 c.c. flask. The residue in the dish is repeatedly extracted with 10 c.c. portions of 96% alcohol, which are decanted into the 100 c.c. flask, until this contains about 95 c.c. The contents of the flask are cooled to 15° and 96% alcohol added until the volume reaches 100 c.c. The liquid is filtered and 90 c.c. evaporated in porcelain on a water-bath, avoiding vigorous boiling of the alcohol. The residue is taken up with a small quantity of absolute alcohol which is poured into a stoppered cylinder, and the dish washed out with more alcohol until the cylinder contains 15 c.c. Three separate portions of absolute ether, each of 7.5 c.c. are now added to the contents of the cylinder, which is vigorously shaken after each addition. When the liquid appears quite clear, it is poured into a tared glass dish, the cylinder rinsed with 5 c.c. of alcohol-ether mixture (2:3) and the rinsings added to the dish, which is then placed on a hot water-bath, which, however, must not be so hot as to cause actual boiling of the liquid. The syrupy residue is dried in the steam oven for an hour, cooled in a desiccator and weighed.

b. In wines containing more than 2 grm. of sugar per 100 c.c., 50 c.c. are warmed in a capacious flask on the water-bath, and mixed with 1 grm. of quartz sand and small quantities of milk of lime added till the colour, at first dark, again becomes pale and the liquid assumes a characteristic alkaline odour. After cooling, 100 c.c. of 96% alcohol is added, the precipitate allowed to subside, and the liquid filtered,

the precipitate and filter being washed with 96% alcohol. The filtrate is then treated as in process (a).

The above troublesome process is likely to remain official until either an exact method is evolved or a simpler method devised which will give results strictly comparable with those obtained by the ether-alcohol method. Windisch (loc. cit., 80-82) gives a complete set of references to the literature of the subject up to 1895. Since then the most important communication has been that of Trillat (Compt. rend., 1902, 135, 903) who recommends the following simple method, which has some followers in France.

50 c.c. of wine are evaporated to one-third bulk in a silver or nickel dish at 70°, 5 grm. of animal charcoal is added, and evaporation continued to dryness, when the residue is mixed in a mortar with 5 grm. of quicklime, extracted twice by thorough shaking in a flask with 30 c.c. of dry ethyl acetate (free from alcohol) and the extract filtered, evaporated on the water-bath, and dried in an oven at 60° to constant weight.

Glycerol itself is none too easily extracted by ethyl acetate, and in consequence other workers (Rocques, Ann. Chim. anal., 1905, 10, 306, and Billon, Rev. intern. falsif., 1906, 19, 57) have improved on Trillat's method until it is quite as complicated as the ether-alcohol method.

An entirely original method is that of Laborde (J. Pharm., 1895, 6, 1, 568, and Ann. Chim. anal., 1899, 4, 76 and 110, and 1905, 10, 340). It is based on the fact that at 150° to 200° glycerol is quantitatively decomposed by sulphuric acid with the liberation of the whole of the carbon which Laborde weighs as such.

As one of the newer methods may obtain official sanction if experience shows it to be quicker and to give results comparable with those obtained by the present official methods, it has been thought worth while to bring Windisch's list of references up to date, following his classification. In addition to the methods already referred to, the following have been described since 1895:

1. Modifications of the ether-alcohol method.
   Fabris, L'Orosi, 1897, 20, 260. Details for manipulation of sweet wines only.
   Guglielmetti and Copetti, Ann. Chim. anal., 1904, 9, 11.

2. Oxidation Methods.
   Depending on the use of potassium permanganate in acid

solution.   Mancuso-Lima and Scarlata, *Staz. Sper. Agrar.*, 1895, **28**, 206.

Depending on the use of chromic acid.   Bordas and de Raczkowski, *Compt. rend.*, 1896, **123**, 1021.

3. By esterification.

As triacetin.   Bottinger, *Chem. Zeit.*, 1897, **21**, 659.

4. By steam-distillation under reduced pressure.   Bordas and de Raczkowski, *Compt. rend.*, 1897, **124**, 240.

5. By conversion into isopropyl-iodide by Zeisel's method, weighing as silver iodide.

Zeisel and Fanto, *Zeit. anal. Chem.*, 1903, **42**, 549.
(See under *Glycerol*, Vol. II.)

Extract.—In sweet wines, sugar forms an important part of the solids, the extract can be ascertained with fair accuracy from the sp. gr. of the original wine and that of the alcoholic distillate, on the assumptions, not very inaccurate, that a solution of wine solids, containing 10 grm. per 100 c.c., has a sp. gr. of 1.0386 and that the excess gravity over water ($=1$) is proportional to the amount of extract present. If s be the sp. gr. of the wine, and s' the sp. gr. of the alcoholic distillate diluted to a bulk equal to that of the wine from which it is derived, then the extract, x, in grm. per 100 c.c., is given by:
$$x = (s - s') \div 0.00386.$$

If the extract so determined is less than 5 % a direct estimation is advisable, as the factor 0.00386 is much less accurate for the other wine solids than for the sugar, which predominates only in wines of relatively high extract content.   To this end 25 c.c. (or 50 c.c. if the extract is below 2.5 %) are evaporated to a thick syrup in a 3-in. flat-bottomed platinum dish, transferred without delay from the water-bath to a steam oven, and after 2 hours, cooled in a desiccator and weighed.   If the extract much exceeds 5 % it is better to be satisfied with the indirect estimation from the sp. gr., since it is practically impossible to dry such extracts under the above conditions.   The direct estimation in any case is purely empirical, the result depending on the size and thickness of the dish and the size, shape and manner of ventilation of the steam oven; all these are rigidly defined by continental workers.

In Germany no direct estimation is made if the extract exceeds 4 %; if it is less than 3 % 50 c.c. are evaporated, while if it is between

3 and 4%, so much is evaporated as will leave not more than 1.5 grm.

The official United States directions are to take 50 c.c. for dry wines and 25 c.c. for sweet wines, with the reservation that when the extract exceeds 6% no direct estimation is to be attempted.

In France extract is estimated by evaporation in a dish as described, followed by 6 or 7 hours heating on the water-bath. This method gives lower results, since most of the glycerol is expelled, whereas during 2 hours in the oven little is lost.

Ash.—The residue from the estimation of extract, or from the evaporation of 25 c.c. of the wine if the extract was not directly determined, is cautiously charred, and repeatedly extracted with small portions of hot water, which are then decanted through a small ashless filter. The filter is then returned to the dish, dried and ashed. When the ash is quite white, the filtrate is added to the contents of the dish, evaporated to dryness, moistened with ammonium carbonate solution, heated to a dull redness, cooled in a desiccator and weighed.

Total Acid.—25 c.c. of the wine are quickly heated to incipient boiling and quickly titrated with N/2 sodium hydroxide, using litmus paper as indicator. The alkali should be standardised against 25 c.c. of a N/10 solution of an organic acid under the same conditions and with the same indicator. The object of heating is not only to expel carbon dioxide, but to reduce the amphoteric reaction of the wine when nearing neutralisation. If the liquid be heated quickly and only until it begins to boil no appreciable loss of acetic acid will result. The use of N/2 alkali reduces the bulk of cold liquid to be added and hastens the titration; the reading is smaller than with N/10 alkali, but is sufficiently large. The acidity is usually calculated as tartaric acid, though in fact free tartaric acid is seldom present in more than traces.

In France the convention is to calculate the acidity as equal to so many grm. of sulphuric acid per 1000 c.c. and it is important to bear this definition of acidity in mind when referring to French standards.

In the United States official methods 25 c.c. of wine are, after shaking to expel carbon dioxide, titrated cold with N/10 sodium hydroxide, using as indicator litmus solution, or in the case of red wines the change of the wine colour to violet.

Volatile Acid.—50 c.c. of the wine are distilled in a current of steam free from carbon dioxide. A little tannin added to the wine prevents - foaming and is preferable to the use of a spray trap. The wine is

directly distilled until the volume is reduced to about 25 c.c.; steam is turned on 'and the flame under the flask so adjusted that the volume remains about 25 c.c. while a total distillate of 200 c.c. is collected. This is titrated with N/10 sodium hydroxide, using phenolphthalein as indicator, and the acidity calculated as acetic acid.

Fixed Acid.—This is estimated by difference. Since the total acid was reckoned as tartaric and the volatile as acetic acid, the fixed acid in terms of tartaric acid is found by subtracting 1.25 times the volatile acid from the total acid.

It is not permissible to estimate the fixed acids directly by evaporating to dryness, subsequently titrating the redissolved residue, since fixed acids may be destroyed during the last stages of the evaporation. On the other hand, Windisch (*Zeit. Nahr. Genussm.*, 1905, 9, 70) has shown that the above-described method for the estimation of volatile acids is not quite satisfactory on account of the partial volatility of lactic acid, which may be the chief acid present, and he suggests the following method. 25 c.c. of the wine are evaporated to about 3 c.c., 25 c.c. of hot water added, and the liquid again evaporated to 3 c.c., and this process repeated once more. The residue is diluted and titrated with standard alkali. From the result is calculated the fixed acid and from this and the total acid the amount of volatile acid may be obtained indirectly.

Reducing sugar is estimated with Fehling's solution. Two hundred c.c. of wine are neutralised with sodium hydroxide and evaporated to about 50 c.c., cooled, transferred to a 200 c.c. flask and diluted to about 160 c.c. Basic lead acetate solution (20 c.c.) is added and the contents of the flask made up to the 200 c.c. mark with water. The mixture is shaken and filtered. To 100 c.c. of the filtrate, 10 c.c. of a saturated solution of sodium sulphate are added, the mixture shaken and filtered. The filtrate, a volume of 11 c.c. of which corresponds to 10 c.c. of wine, serves for the estimation of reducing sugars. This may be carried out as described in the "Sugars" section of this work. In Germany and the United States, the gravimetric method is officially practised, 25 c.c. of the above filtrate being taken for the test. In Germany 50 c.c. of Fehling's solution and 25 c.c. of water are taken, the precipitate reduced and weighed as copper in a Soxhlet tube, and calculated as invert sugar by means of Wein's tables. In the United States 60 c.c. of Fehling's solution and 60 c.c. of water are taken and the precipitate oxidised to CuO in a Gooch crucible, the result being

calculated from Allihn's tables as dextrose. The volumetric method, using ferrous thiocyanate as indicator (Ling and Rendle, *Analyst*, 1905, **30**, 182) is much quicker and, according to Ling and Jones (*Analyst*, 1908, **33**, 160), quite as accurate.

Note.—In order that results by the gravimetric method may be calculated by aid of the published tables, it is necessary that the amount of copper or oxide to be weighed shall fall within certain limits. If the sugar content does not exceed 1 grm. per 100 c.c. the above quantities will give a convenient precipitate. If the proportion of sugar is greater than 1 % a smaller quantity of wine should be taken in the first instance. The sugar content is as a rule not very far from x — 2, where x represents the grm. of extract per 100 c.c. If therefore a wine shows 4 % extract, it will probably contain about 2 % of sugar, and 100 c.c. is then diluted with an equal bulk of water, evaporated down to about 50 c.c., cooled and made up to 200 c.c. with lead acetate, etc., as already described.

Cane Sugar.—50 c.c. of the clarified solution taken for the estimation of reducing sugars are exactly neutralised with hydrochloric acid, 5 c.c. of 1 % hydrochloric acid added, and the whole heated for half an hour on the water-bath. The liquid is exactly neutralised, evaporated somewhat, made slightly alkaline with sodium carbonate and filtered into a 50 c.c. flask, the filter being washed until the flask is full to the mark. The reducing power of this filtrate is now determined by means of Fehling's solution, and the result calculated as invert sugar. As 95 parts of cane sugar yield 100 parts of invert sugar on hydrolysis, the amount of cane sugar in 100 c.c. of the wine is given by $x = 0.95$ ($b-a$), where a is the amount of reducing sugar, expressed as invert, in 100 c.c. of the original wine, and b the amount found after inversion.

Polarisation.—The polarimeter may give useful information concerning a sample of wine, especially one suspected of sophistication. The following scheme may be found useful. It remains nearly in the words of a Bulletin of the A. O. A. C., from which it was copied into the last edition of this work. As there was one serious mistake the present writer has compared the whole scheme with the German decree (*Veröffent. d. kaiserl. Gesundheitsamtes*, 1896, **20**, 557) on which it was based and has made some minor alterations in the direction of the German model.

All results are to be stated as the polarisation of the undiluted wine in a 200 mm. tube. The Schmidt and Haensch half-shadow saccharimeter is to be used, and the results expressed in terms of the sugar

scale of this instrument. If any other instrument be used, or if it be desirable to convert to angular rotation, the factors given on page 53 are to be used.

White Wines.—60 c.c. of wine are neutralised, evaporated to one-third, made up again to 60 c.c., treated with 3 c.c. of basic lead acetate solution and filtered. 31.5 c.c. of the filtrate are treated with a 1.5 c.c. of a saturated solution of sodium carbonate, filtered, and polarised. This gives a dilution of 10 to 11, which must be considered in the calculation, and the polarimeter reading must accordingly be increased one-tenth.

Red Wines.—60 c.c. of wine are neutralised, evaporated to one-third, made up again to 60 c.c., decolourised with 6 c.c. of basic lead acetate solution and filtered. To 33 c.c. of the filtrate 3 c.c. of a saturated solution of sodium carbonate are added, the mixture filtered, and the filtrate polarised. The dilution in this case is 5 to 6, and the polarimeter reading must accordingly be increased one-fifth.

Sweet Wines, Before Inversion.—100 c.c. are neutralised, evaporated to one-third, made up again to 100 c.c., decolourised with 2 c.c. of basic lead acetate solution and filtered after the addition of 8 c.c. of water. 0.5 c.c. of the saturated solution of sodium carbonate and 4.5 c.c. of water are added to 55 c.c. of the filtrate, and the liquid mixed, filtered, and polarised. The polarimeter reading is multiplied by 1.2.

After Inversion.—33 c.c. of the filtrate from the lead acetate in (1) are placed in a flask with 3 c.c. strong hydrochloric acid. After mixing well the flask is placed in water and heated until a thermometer, placed in the flask with the bulb as near the centre of the liquid as possible, marks 68°, consuming about fifteen minutes in the heating. It is then removed, cooled quickly to room temperature, filtered, and polarised, the temperature being noted. The polarimeter reading is multiplied by 1.2.

After Fermentation.—50 c.c. of wine, are dealcoholised and made up to the original volume with water, and mixed in a small flask with well-washed beer yeast and kept at 30° until fermentation has ceased, which requires from two to three days. The liquid is then washed into a 100 c.c. flask, a few drops of a solution of acid mercuric nitrate and then basic lead acetate solution, followed by sodium carbonate, added. The flask is filled to the mark with water, shaken, and the solution filtered and polarised.

(1) *The Wine Shows No Rotation.*

This may be due to the absence of any rotatory body or to the simultaneous presence of dextrorotatory and laevorotatory sugars.

(a) *The Wine is Inverted.*—A laevorotation shows that the sample contained cane sugar.

(b) *The Wine is Fermented.*—A dextro-rotation shows that both laevorotatory sugar and the unfermentable constituents of commercial dextrose were present.

If no change takes place in either (a) or (b) in the rotation it proves the absence of unfermented cane sugar, the unfermentable constituents of commercial dextrose, and of laevorotatory sugar.

(2) *The Wine Rotates to the Right.*

This may be caused by unfermented cane sugar, commercial dextrose, or both.

The Wine is Inverted.

(a_1) *It Rotates to the Left After Inversion.*—Unfermented cane sugar was present.

(a_2) *It Rotates More than 2.3° to the Right.*—The unfermentable constituents of commercial dextrose are present.

(a_3) *It Rotates Less than 2.3° and More than 0.9° to the Right.* —It is in this case treated as follows:

210 c.c. of the wine are evaporated to about one-third volume to expel alcohol, cooled, diluted with water to the original volume, and fermented with 2 grm. of pressed yeast. The fermented liquid is evaporated in a porcelain dish to a thin syrup with a little sand and a few drops of a 20 % solution of potassium acetate. To the residue 200 c.c. of 90 % alcohol are added, with constant stirring. The alcoholic solution is filtered into a flask, and the alcohol removed by distillation until about 5 c.c. remain. The residue is mixed with washed boneblack, filtered into a graduated cylinder, and washed until the filtrate amounts to 30 c.c. If the filtrate shows a dextro-rotation of more than 1.5° it indicates the presence of the unfermentable constituents of commercial dextrose.

(3) *The Wine Rotates to the Left.*

It contains unfermented laevorotatory sugar, derived either from the must or from the inversion of added cane sugar. It may, however,

also contain unfermented cane sugar and the unfermentable constituents of commercial dextrose.

(a) The wine is fermented according to the process already described.

(a₁) It polarises 3° after fermentation. It contains only laevo-rotatory sugar.

(a₂) It rotates to the right. It contains both laevorotatory sugar and the unfermentable constituents of commercial dextrose.

(b) The wine is inverted according to the process already described.·

(b₁) It is more strongly laevorotatory after inversion. It contains both laevorotatory sugar and unfermented cane sugar.

Potassium Sulphate.—Fifty c.c. of the original wine is acidified with hydrochloric acid, precipitated hot with barium chloride and the sulphate found calculated as potassium sulphate.

Sulphurous Acid.—The sulphuring of casks is a common and not improper practice, but the presence of a considerable amount of sulphurous in wine indicates that sulphites have been added as preservative. Some of this sulphurous acid is combined with aldehyde. and since in this form it is said to be less objectionable (Marischler, *Wien. klin. Wochenschr.*, 1896, 31), it is usual to make two estimations, one of total sulphurous acid, the other of sulphurous acid not in organic combination, which latter is in contradistinction described as "free" sulphurous acid.

"Free" Sulphurous Acid.—To 50 c.c. of the wine, contained in a flask, a little sodium carbonate is added and then excess of dilute sulphuric acid. The flask is thus filled with carbon dioxide and the sulphurous acid can be titrated fairly accurately with N/50 iodine solution and starch.

Total Sulphurous Acid.—50 c.c. of the wine are mixed in a flask with 25 c.c. of normal sodium hydroxide to liberate the sulphurous acid from its combination with aldehyde. After the mixture has stood 15 minutes with occasional shaking, 10 c.c. of dilute (1:3) sulphuric acid are added, and the total sulphurous acid quickly titrated with N/50 iodine solution.

More exact but more complicated methods for the estimation of total sulphurous acid may be found in Windisch (page 133), Bigelow (page 57) and in *J. Soc. Chem. Ind.*, 1898, **17**, 279. If the amount of sulphur dioxide permissible is fixed by law the use of an exact

method is important. There is a tendency for the amount to be slightly overestimated by the method just described, but it is sufficiently accurate for most purposes.

Tannin.—The following approximate method, due to Nessler and Barth (*Zeit. anal. Chem.*, 1883, **22**, 595) has many followers on the continent. 12 c.c. of wine are shaken with 30 c.c. of 96% alcohol and filtered. 35 c.c. of the filtrate, corresponding to 10 c.c. of wine, are evaporated to 6 c.c. and transferred to a measuring tube of prescribed dimensions, the volume brought up to 10 c.c. by addition of water, 1 c.c. of 40% sodium acetate added and finally 1 or 2 drops of 10% ferric chloride. The whole is t hen shaken and after 24 hours the volume of the precipitate is read off. This volume in c.c. multiplied by 0.033 is said to give the approximate percentage of tannin in the wine. The lower part of the measuring tube is 0.8 cm. wide and shows tenths of a c.c., and is long enough to hold about 4 c.c.; the upper part is 1.8 cm. wide and has marks at 10, 11, 20 and 22 c.c. With red wines, it is usual to add 11 c.c. of water immediately after the addition of the ferric chloride and before shaking.

For more exact work, Neubauer's modification of Löwenthal's method (see Tannins, Vol. III) is most often employed. 10 c.c. of wine is a convenient quantity to take for the process.

Salicylic Acid.—This properly belongs to another section of this work. Since, however, genuine unsophisticated wine may give the reactions of salicylic acid, if large enough quantities are taken for the test, the German official directions for carrying out the test are given here. It is said that few or no genuine wines will give the characteristic reactions of salicylic acid when the test is conducted with these quantities, whereas added salicylic acid will be infallibly detected, since a quantity undetectable in this way would not appreciably increase the keeping properties of wine.

50 c.c. of wine are shaken (not too vigorously, lest an emulsion forms) with 50 c.c. of a mixture in equal proportions of ether and petrolleum spirit. The ethereal layer is separated, filtered and evaporated, and the residue tested with very dilute ferric chloride solution. The tannin is almost insoluble in the mixture of ether and petroleum, but if a black or dark brown colour result on the addition of ferric chloride, a drop of hydrochloric acid is added and the extraction with the solvent repeated.

Saccaharin.—In the absence of salicylic acid, any of the methods

described under *Saccharin* in a later volume of this work may be applied. Methods depending on the conversion of saccharin into salicylic acid are clearly not applicable when salicylic acid is present.

The following method, due originally to Herzfeld and Reischauer (*Deutsche Zuckerind.*, 1886, 124), was especially recommended by Allen (*Analyst*, 1888, **13**, 105):

100 c.c. of wine are mixed with coarse sand and evaporated on the water-bath. The residue is treated with 1 or 2 c.c. of 30 % phosphoric acid and repeatedly extracted with a moderately warm mixture, in equal proportions, of ether and petroleum light. The successive extracts, which should amount in all to 200 c.c. or more are filtered through asbestos and the greater part of the solvent removed by distillation. The concentrated extract is poured into a basin, the remainder of the solvent evaporated, and the residue taken up with dilute sodium carbonate solution, filtered into a platinum dish and evaporated to dryness.

The residue is mixed with 4 or 5 times its bulk of dry sodium carbonate and added in small portions to fused nitre. The melt is dissolved in water, acidified with hydrochloric acid and precipitated with barium chloride. Each grm. of barium sulphate corresponds to 0.786 grm. saccharin.

Boric Acid.—Methods of estimating this need no description here, but it may be pointed out that merely qualitative tests are valueless, since it is now known that boric acid is a normal constituent of wines.

Fluorides.—For the estimation of fluorides in wine, Treadwell and Koch (*Zeit. anal. Chem.*, 1904, **43**, 469) recommend the following modification of Rose's method. 100 c.c. of the wine are rendered feebly alkaline with sodium hydroxide, and silver nitrate is added as long as it produces a precipitate. The mixture is then made up to 250 c.c., filtered, and 200 c.c. of the filtrate treated with an excess of sodium chloride, and made up to 250 c.c. After 24 hours, 175 c.c. of the clear liquid are decanted off and treated with 3 or 4 c.c. of 2N sodium carbonate, and then boiled for five minutes with a large excess of calcium chloride. The precipitate is collected on a filter, washed with hot water, dried and heated to dull redness for 15 minutes. When cool, 3 c.c. of 9% acetic acid are added to the contents of the crucible and the whole digested on the water-bath for half an hour, after which the mixture is evaporated to dryness. Two drops of 9 %

acetic acid are added, and the residue in the crucible repeatedly extracted with small portions of hot water, which are then passed through a small filter. This is subsequently washed, dried, ashed and returned to the crucible and the latter ignited and weighed. Extraction with acetic acid should be repeated until two weighings, differing by less than 0.5 mg. are obtained. 1.6 mg. are added to the weight for each 100 c.c. of wash-water used.

Occasionally, but rarely, chlorine, lime, magnesia, and potash are estimated in wines. The methods for their estimation need no description here.

For the estimation of total tartaric acid, free tartaric acid, potassium hydrogen tartrate, and calcium tartrate, Halenke and Möslinger (*Zeit. anal. Chem.*, 1895, **34**, 279) have worked out an exact scheme of analysis, which is described very fully by Windisch (*loc. cit.*, 120). These determinations are apparently considered of importance by German officials, since the methods of Halenke and Möslinger are set out in some detail in the Imperial decree to which reference has already been made. The results will have more significance when a larger number of genuine wines have been examined by the accurate methods to which reference has been made. Most of the published statistics are based on older and less exact methods of analysis.

Foreign Colouring Matters.—Wines should always be examined for the presence of coal-tar colours, and tested as to their behaviour with lead acetate. Even white wines may have received an addition of caramel or of a coal-tar colour specially prepared as a caramel substitute. Some cleaned wool, mordanted with alum and sodium acetate, should be boiled with the wine and any precipitated colour examined by the usual reagents (see Vol. V, and Green, *J. Soc. Dyers and Col.*, 1905, **21**, 236). It is usually sufficient to establish the presence of such colours, however, and not necessary to identify them.

The following tests have been specially recommended for the detection of foreign colouring matters in wine.

Lead Acetate Test.—5 c.c. of basic lead acetate solution are added to 20 c.c. of wine. If the resulting precipitate is red-violet in colour, the fact is strong evidence of the presence of the colouring matter of poke berries (*Phytolacca decandra*). Bilberry juice gives a blue precipitate and mallow and elderberry juice a green one, but these colours are much less characteristic than that given by poke. Gen-

uine wines may give a grey, blue-grey, blue-green or green precipitate, but never a red-violet one.   Another volume of 5 c.c. of lead acetate is added and the liquid warmed and filtered.   If the filtrate is red, rosaniline may be suspected, but some genuine dark red wines are only with difficulty decolourised by basic lead acetate.   If amyl alcohol, shaken with the filtrate, assumes a red colour the presence of artificial colouring matters may be inferred with certainty.

Wool Test.—White wool, mordanted with alum and sodium acetate, is boiled with the wine to which 10% of its bulk of 10% potassium sulphate has been added.   Genuine wines may impart a red colour to the wool which remains after washing, but this colour is much less intense than that given by minute traces of coal-tar dyes and may be distinguished by its turning a dirty greenish-white on treatment with ammonium hydroxide.   If the colour is of coal-tar origin, it will remain unchanged or change to a yellowish tint, which reverts to red on washing out the ammonia.

Cazeneuve's Mercuric Oxide Test.—10 c.c. of wine are shaken with 0.2 gr. yellow mercuric oxide for at least a minute, and when the oxide has completely settled the liquid is filtered.   Several thicknesses of paper are sometimes necessary and if a clear filtrate cannot be obtained in this way, the experiment should be repeated and the mixture heated to boiling before shaking.   A clear but coloured filtrate indicates the presence of coal-tar colours, but a colourless one is no proof of their absence, since many, including the rosanilines, are absorbed by mercuric oxide.   The test serves, however, to detect acid fuchsin, Bordeaux red, and other colours which escape the test with basic lead acetate.

Shaking with Ether Before and After Supersaturation with Ammonium Hydroxide.—To 100 c.c. of wine, 5 c.c. of ammonium hydroxide are added, and the mixture shaken with 30 c.c. of ether.   Another 100 c.c. of wine are shaken with ether without the addition of ammonia.   From each ethereal layer, 20 c.c. are withdrawn with a pipette and allowed to evaporate in a basin containing a thread of wool about 2 inches long.   If the wool from the experiment in which the ammonia was used is dyed red, the presence of coal-tar colours may be inferred.   With genuine wines the wool from the experiment in which ammonia was used remains perfectly white, while that from the experiment without ammonia usually acquires a brownish tint.   The test serves for the detection of rosanilin, safranin and chrysoïdin,

but acid fuchsin and many other colours which may be present are not detected by it.

Shaking with Amyl Alcohol.—This test is best performed in triplicate: (a) on the original wine; (b) on the wine made acid with sulphuric acid, and (c) on the wine made alkaline with ammonia. One hundred c.c. of wine and 30 c.c. of amyl alcohol are convenient quantities.

a. If the amyl alcohol is coloured red the presence of artificial colouring matter is not necessarily to be inferred, since many high-coloured young wines yield red colouring matter to amyl alcohol. If on the addition of a few drops of ammonia the colour remains unchanged, the presence of coal-tar colours is tolerably certain, since the red colour of genuine wines is changed to blue or green on such treatment.

b. The amyl alcohol extract from acidified red wines is generally red, but any artificial colour is concentrated by the treatment and separated from some other matters which may mask its reactions. The amyl alcohol is shaken with water and the aqueous solution tested with ammonia or submitted to the wool test.

c. If the amyl alcohol extract from the ammoniacal wine is red, the presence of coal-tar colours may be safely inferred. If it is colourless the experiment should be repeated, using less ammonia since in presence of a large excess (above 3 %) of ammonia the amyl alcohol may remain colourless even when coal-tar colours are present.

The detection of *caramel* in wine is less important now than it was some years ago, since tar colours have been specially prepared to replace it, and the necessary quantity of these tar colours is so small that their expense is negligible, and the sophisticator probably thinks them less easy of detection. In point of fact, the time-honoured tests for caramel fail to detect modern preparations sold under this name. This is not surprising since the caramel of to-day is a widely different product from the burnt sugar of twenty years ago; it contains a notable proportion of amino-compounds and is chemically very different from earlier preparations, in the published analyses of which nitrogen was never recorded. The white-of-egg test of Carles (*J. Pharm. Chim.*, 1875, **22**, 177) remains in all the text-books, although a liquid coloured by a modern preparation of caramel loses quite as much colour as many genuine wines on treatment with white of egg. Schidrowitz (*J. Soc. Chem. Ind.*, 1902, **21**, 816) has doubly discredited Amthor's paralde-

hyde test (*Zeit. anal. Chem.*, 1885, **24**, 30) which may discover caramel where there is none and fail to discover it when actually present.

In the writer's experience, however, caramel will be readily enough detected by the general tests already given. Contrary to the statements in the text-books, caramel, of English manufacture at least, is not decolourised by basic lead sub-acetate so that this test will show the presence of foreign colouring matter of some sort. The comparative insolubility of the colour in amyl alcohol and its almost complete insolubility in ether will serve to distinguish it from coal-tar colours if it is necessary to do this.

The foregoing tests have many years' successful application to recommend them. No single one of them will carry the analyst very far, but if they are all applied, few artificially coloured wines will escape detection. Those few will be coloured with bilberry juice or similar fruit juice, and the methods described for the detection of bilberry juice are so troublesome and withal so uncertain that they are scarcely worth description here.

New methods for the detection of foreign colouring matters, especially of magenta, in wines are described each year, and occasionally the claim is made for a new test that it enables the analyst by a single operation to decide whether a sample has been artificially coloured or not. The continued use of the older, more troublesome methods by experienced analysts must be taken as evidence that the comprehensiveness of the new tests yet lacks proof.

One of the best of recent suggestions is that of Jean and Frabot (*Ann. chim. anal.*, 1907, **12**, 52, and *Bull. Soc. Chim.*, 1907, **1**, 748). Extending some experiments of Trillat, these authors find that all genuine wines yield a colourless filtrate when treated as follows: 50 c.c. of the wine is warmed on the water-bath with 1 c.c. of formalin and 4 c.c. of hydrochloric acid. When a precipitate has formed, an excess of ammonia is added and the heating continued till all the free ammonia has been expelled. The liquid is then cooled and filered. They also find that artificially coloured wines when treated in this way yield a coloured filtrate. It is useful to know that all genuine wines which have been tested in this way yield colourless filtrates, but it would be unwise to regard such a colourless filtrate as proof that no colour other than that natural to the wine was present.

The following method, due to Dupré (*J. Chem.Soc.*, 1880, **37**, 572), has been useful to many. The best colourless commercial gelatin is dis-

solved in ten parts of boiling water, and the solution poured into a soup-plate or other flat vessel. When cold and thoroughly set, a cube about ¾ in. on the side is cut from the jelly by means of a sharp knife and placed in the sample of wine to be tested. After standing 24 hours, the cube is removed, washed a little with cold water, and a central slice cut out of it in a direction parallel to one of the sides. On examining this section, it will be found, in the case of a pure wine, that the colouring matter has penetrated but a very little way into the jelly (perhaps 1/16 in.), whereas the great majority of foreign colouring matters will have penetrated to the very centre of the cube.

Of a large number of colouring matters only that of *alkanet-root* resembles the "œnolin" of pure wine in the slow rate at which it diffuses into the jelly. Hence, if coloration of the interior of the jelly is not observed, alkanet is the only foreign colouring agent likely to be present. It may be distinguished by its absorption-spectrum, which, at a certain concentration of the acidulated solution, shows three distinct absorption-bands between the sodium line and the blue strontium line, and nearly equidistant from these lines and from each other. Ammonia changes the colouring matter of alkanet to blue, and reduces the absorption-bands to two, one coincident with the D line and the other less refrangible than that. Both acid and alkaline solutions produce a general absorption of the violet end of the spectrum, and in moderately concentrated solutions only the red is transmitted.

The colouring matter of pure red wine produces a general absorption in all parts of the spectrum except the red, but generally no distinct absorption-band. The red colour is changed to greenish-brown on addition of ammonia, and the liquid then shows an indistinct absorption-band in the orange-yellow region.

If the colouration of the cube of jelly points to the presence of a foreign colouring matter, the nature of this may frequently be ascertained, if desired. As a rule, the slice of jelly shows the colour proper to the added substance much more clearly than did the wine itself, and a difference between the two colours is a strong indication of the presence of a foreign matter. *Indigo* and *logwood* may thus be readily discovered. The absorption-spectrum exhibited by the slice will serve for the detection of rosaniline, cochineal, beet-root, red-cabbage, litmus etc., and further information may be gained by placing the slice in dilute ammonia. Thus treated, a slice coloured with *rosaniline* becomes colourless; with *red cabbage*, dark green; with *cochineal*, purple, and with *logwood*,

brown. This last reaction is, however, frequently produced in the absence of logwood. When present, the slice will be coloured brown or yellow to a considerable depth before it is treated with ammonia.

Operating in the above manner, Dupré found that an addition of foreign colouring matter equal to 10 % of the total intensity of the colour of the wine could usually be readily detected, and in no case could 20 % be overlooked. In the case of logwood 5 % could be recognised, and as little as 1 % of rosaniline could be found. In making the tests it is desirable to compare the sample with a pure wine of the same kind.

SIGNIFICANCE OF RESULTS OF WINE ANALYSIS.

The chemist who needs to refer to a general work of this kind for analytical methods will presumably only seek to know how he may distinguish genuine wine from sophisticated beverages. The significance of the results to the owner or intending purchaser of wine of undoubted genuineness but doubtful capacity for improvement on keeping cannot be dealt with here, experience of the wines of a particular district and the possession of a trained palate are indispensable to the formation of a sound judgment of the future behaviour of a wine.

The following notes assume that the analyst is concerned only in deciding as to the genuineness or otherwise of a sample. Thus the alcoholic content is described as of small significance, which from this standpoint is true, but to a wine expert a difference of 2% may suggest a great deal as to the relative stability of two wines.

The standards most respected in France and Germany are applicable only to wines which should be the product of fermentation of normal grape-musts, without concentration of these musts or addition of alcohol, sugar or other substance. They are occasionally subjected to criticism even in the countries of their origin, and it would be unfair to apply them to the wines of other countries. The standards which have been proposed for certain sweet wines are of still narrower application, and there are insufficient data available concerning the wines of Spain and Portugal to justify any standards for the admittedly fortified wines of those countries. Except when otherwise stated, the following notes refer only to wines which, if genuine, are the undiluted product of the fermentation of pure grape-musts, and the word "genuine" when used is to be understood in this sense.

Specific Gravity.—This is of small significance in judging a wine, and the main purpose of ascertaining it is the estimation of extract by the indirect method. The sp. gr. of wines derived by natural fermentation from the juice of the grape, without concentration or addition of any kind, is never far from unity, seldom less than 0.99 and, according to certain French authorities, never less than 0.985. In wines which may properly be derived from concentrated musts or to which the addition of alcohol is a recognised practice, the variations in sp. gr. may be very great.

Alcohol.—The alcohol content of genuine wines usually lies between 5 and 10 grm. per 100 c.c., but numbers as low as 2.1 and as high as 12.2 have been recorded. Alcohol in excess of 14.5 grm. per 100 c.c. would be certain evidence of added spirit, but it must be remembered that even in Germany such addition is permitted by law, provided it does not exceed 0.8 grm. per 100 c.c. of wine. Since the alcohol content of genuine wine may vary so widely, the number is of small value in determining whether a sample has been diluted with water.

Glycerol.—The proportion of glycerol usually lies between 0.4 and 1% but may be as low as 0.16 or as high as 1.4.

Alcohol-glycerol Ratio.—German chemists attach more importance to this number than to the absolute percentages of alcohol or glycerol, and this is reasonable, but the standards set up some years ago require amendment even for German wines. It was formerly supposed that the ratio of alcohol to glycerol in genuine wines always lay inside the limits 100 : 7 and 100 : 14. A wine which showed a higher ratio of alcohol to glycerol than 100 : 7 was held to have been fortified by addition of spirit, while if the ratio fell below 100 : 14, addition of glycerol was suspected. It is now known that in genuine Rhine wines the alcohol-glycerol ratio may exceed 100 : 6 or fall below 100 : 19. If 100 : 5 and 100 : 20 be taken as the limits, few genuine European wines will be excluded, but Bigelow has pointed out that the average alcohol-glycerol ratio for American wines is about 100:6 and in his table are included wines in which it is as high as 100:2.

Extract.—The percentage of sugar varies within wide limits, and plastered wines may contain notable quantities of potassium sulphate. Apart from these two constituents, the percentage of solids in solution in young wines is fairly constant and has been ascertained to be never

less than a certain amount. Unfortunately, French chemists are not content with recording the extract less sugar and potassium sulphate, but, since these latter are normally present in small amount, they define "reduced extract" as $x-(S-0.1)-(K-0.1)$, where x, S and K represent the percentage of extract, sugar and potassium sulphate in the wine. For the purpose of comparison with arbitrary standards the simpler formula $x-S-K$ would serve equally well, but in this and the following paragraphs the expression "reduced extract" is used in the French sense. The reduced extract of genuine white wine of continental origin is seldom less than 1.6 grm. per 1000 c.c. that of red wine seldom less than 1.8 grm. The amount of extract decreases with age, but seldom falls below 1.5 grm. Bigelow quoting M. Curtis, of San Francisco, says that American red wine is to be viewed with suspicion if it contain less than 2.4 or more than 3.3 grm. reduced extract per 1000 c.c. For American white wine he places the limits at 1.5 and 2.4 grm.

Alcohol-extract Ratio.—In France more importance is attached to this number than to the ratio of glycerol to alcohol. It is said that for genuine red wines the ratio never exceeds 4.5, while for white wines it may be higher but is never in excess of 6.5. Higher values are to be taken as proof of added alcohol. This test is more severe when the French method of determining extract is used, since by that method glycerol is largely driven off, and a lower number obtained for the extract.

Ash.—The ash content of wines usually lies between 0.2 and 0.3% but genuine wines have been known to contain as little as 0.11 and as much as 0.44. A smaller amount than 0.14% would justify suspicion, but it is less easy to fix an upper limit, though it may be fairly said that 0.35 is rarely exceeded. The ash follows the reduced extract to some extent, and is higher for red wines. In attempting to draw conclusions from the amount of ash it is well to deduct from this the percentage of potassium sulphate found less 0.1.

Total Acid.—The total acid, calculated as tartaric acid, is seldom less than 0.4 or more than 1.5%. In France the total acid is calculated as sulphuric acid, and it is held that the sum of the alcohol (expressed as c.c. per 100 c.c.) and the acid (calculated as grm. of sulphuric acid per 1000 c.c.) is never less than 12.5 for a genuine wine. A lower value is held to be evidence of dilution with water. This is per-

haps the most frankly empirical standard which has been applied to wines, but it has behind it the experience of a whole generation of French chemists and may presumably be applied with confidence to wines purporting to be of French origin. When the alcohol-extract ratio exceeds 4.5 for red wines or 6.5 for white wines, the "natural" percentage of alcohol is substituted for that actually found. For example, if a red wine contains 12 grm. alcohol and 1.5 grm. reduced extract per 100 c.c., it is obvious that alcohol has been added. This added alcohol must not be taken into account in applying the test for added water. Instead, the extract, 1.5, is multiplied by 4.5 to give the "natural percentage" by weight of alcohol, and then divided by 0.8 to obtain the percentage by volume. To the number so obtained, in this case 8.5, the acidity in grm. per 1000 c.c. is added, and if the sum is less than 12.5 it may be taken as evidence that both water and alcohol have been added.

Volatile Acid.—The volatile acid, calculated as acetic acid, is usually below 0.08 % and wine containing much more than 0.15 would be condemned not as fraudulent, but as unsound. As has been said already, considerable experience is necessary in forming judgments of soundness. Thus a wine high in alcohol might be and remain quite sound although the volatile acid was as high as 0.15 while another poor in alcohol and otherwise deficient might have less than 0.10 per cent. and be quite unmerchantable. A trained palate is of the first importance here.

Sugar.—The sugar content of dry wines is of the order of 0.1 %. In the Paris municipal laboratory it is usual to add together the sugar and twice the alcohol, both expressed as grm. per 100 c.c., and if this sum exceeds 32.5, to decide that the wine has received an addition of alcohol or sugar.

Potassium Sulphate.—The juice of the grape contains sulphates equivalent to perhaps 0.05 % of potassium sulphate and a further amount results from the sulphuring of casks, so that wines on the average contain about 0.1 %. An amount in excess of 0.2 % is held to be evidence of plastering—that is, of the addition of gypsum to the must—a practice which is most common in the sherry district, though not confined to it. It is impossible to discuss here the complex reasons for the practice or the arguments which have been brought against it by hygienists. Red wines, except sweet dessert wines, must not in Germany contain more than 0.2% of potassium sul-

phate, and similar regulations apply to the sale of wines in France and Switzerland.

Sulphurous Acid.—In France and Switzerland, sulphurous acid in excess of 200 mg. per 1000 c.c. is forbidden, whilst the "free" sulphurous acid must not exceed 30 mg. per 1000 c.c. in France or 20 mg. per 1000 c.c. in Switzerland.

Most of the work in connection with wine has been carried out in Germany and France, and the standards suggested by French and German chemists are strictly applicable only to the wines typical of those countries, the red and white Bordeaux wines, and hocks. All these are or should be natural wines. The same standards may with a considerable amount of caution be applied to the wine of any other country if that wine purports to be a natural wine. But wines are seldom so labelled; they have a distinctive name. The word "claret" has a perfectly definite significance in England. The purchaser expects a wine grown in the Bordeaux district, treated in the manner usual in that district and having the character common to wines of that name. The analyst would not pass as genuine claret a sample which showed signs of being fortified or heavily plastered. Suppose, however, the sample is sold as "Spanish claret," and proves to be fortified and heavily plastered. The typical wine largely exported from Spain is sherry, which is always fortified and always plastered, and it might be argued with some truth that fortifying and plastering, though they found their highest development in the manufacture of sherry, were not restricted to sweet wines but were more or less typical of Spanish practice. Certainly wines of every degree of sweetness and alcoholic strength from a typical claret up to something indistinguishable from sweet port are sold in Spain itself under one name. Such a note of warning is necessary, as wines of every type are now being produced in four continents, and it is doubtful if the purchaser of Australian burgundy or Californian sauterne has any right to expect more than a reasonable resemblance to previous consigments bearing the same label.

Port, sherry and other Spanish wines less frequently imported to this country, marsala and many Italian wines, and madeira always receive an addition of alcohol to arrest fermentation, and cane sugar is a normal addition to sparkling wines. No champagne maker would risk his valuable crop by using anything but refined cane sugar, but in Germany it has been found necessary to institute penalties to prevent the use of commercial glucose.

CIDER.

The literature of cider continues to grow, French and American chemists being responsible for most of the work, but no special analytical methods of importance have been described. Most of the methods applied to wines may be extended to cider. It is usual to calculate the non-volatile acid as malic acid and to return it as such. The statement which appears in the text-books, for example in the last edition of this work, that the solid matter of cider differs from that of wine in the presence of malic acid, seems to rest on this convention rather than on the results of analyses directed to the differentiation of malic and tartaric acids. A method for the differentiation of malic, tartaric and succinic acids in wines, etc., has been worked out by Schmit and Hiepe (*Zeit. anal. Chem.*, 1882, 21, 534) and may be found described in Windisch (*loc. cit.*, 185). The method is said to be accurate, but it is very tedious and of doubtful utility. So far as it has been applied to wines, the results seem to indicate that malic acid may be the chief constituent of the non-volatile acid of grape wines as well as of cider.

In the Paris municipal laboratory it is held that dry cider—that is, cider containing less than 1% sugar—should not contain less than 3% of alcohol by volume. In judging sweet cider, the sugar percentage less 1 is divided by 2 and then by 0.79 and the number so obtained added to the actual alcohol percentage. The same authority fixes the minimum extract percentage of genuine cider at 1.8, and the minimum ash at 0.17%. This latter figure should be 0.15 or less for English cider, whilst a higher alcohol percentage, say 4, might not unreasonably be insisted on. Information on the manufacture of English cider may be found in a pamphlet by F. J. Lloyd, published by the Board of Agriculture in 1903.

POTABLE SPIRITS.

The estimation of alcohol in potable spirits does not call for special consideration here. It is usually estimated from the sp. gr. of the distillate on the assumption, not quite correct, that the distillate consists solely of water and alcohol. The error introduced by this assumption is, with most spirits, very small.

Higher Alcohols.—The only method which can be recommended is the Allen-Marquardt method. Two others must, however, be de-

scribed in some detail, partly because they have official sanction in certain foreign countries, but more particularly because the analyst may be asked by his clients to apply these specific tests in order that the results may be compared with older records or with the numbers returned by a continental chemist to whom the same sample has been submitted.

Allen-Marquardt Method.—The following description differs slightly from that given by Allen in the last edition of this work, in that certain suggestions of Schidrowitz (*J. Soc. Chem. Ind.*, 1902, 21, 815) have been adopted. To 200 c.c. of the sample about 1 c.c. of strong potash solution is added and the whole boiled for an hour under a reflux condenser. The liquid is then transferred to a distilling flask through the cork of which there passes, to within a few mm. of the bottom of the flask, a tube for the introduction of steam. Before connecting up with the supply of steam, distillation is commenced by the use of an ordinary gas-burner and continued till only about 20 c.c. is left. Steam is then turned on and the flame under the flask so regulated that the contents of the same are reduced to about 10 c.c. by the time 300 c.c. in all have passed over. The distillate is divided into two equal parts and each is treated in the following manner, thus giving a duplicate determination of the higher alcohols:

A saturated solution of common salt is added to the liquid until the resulting mixture has a sp. gr. of at least 1.1, when it is extracted in a separator four times with carbon tetrachloride, using 40 c.c. of the tetrachloride for the first extraction, 30 c.c. for the second, 20 c.c. for the third, and 10 c.c. for the last extraction. The carbon tetrachloride now contains all the higher alcohols, and some ethyl alcohol. To remove the latter, the carbon tetrachloride is shaken with 50 c.c. of brine, and after this has been separated it is shaken with 50 c.c. of a saturated solution of sodium sulphate to remove the chloride. The carbon tetrachloride is next treated with an oxidising mixture consisting of 5 grm. of potassium dichromate, 2 grm. of strong sulphuric acid, and 10 c.c. of water. The oxidation is carried out in a flask which is connected to a reflux condenser, the liquid being kept gently boiling by means of a water-bath for at least eight hours. Any higher alcohols extracted by the carbon tetrachloride will by this treatment be converted into their corresponding acids. After oxidation, the liquid is diluted with 30 c.c. of water, and distilled over a naked flame until only 20 c.c. remain in the flask, which is provided with a tube for the in-

troduction of steam as in the first distillation. Steam is now turned on, and the flame under the flask so regulated that not much more than 5 c.c. remains when the total distillate measures 300 c.c. Distillation is then stopped and the distillate titrated with N/10 barium hydroxide, using methyl-orange as the indicator, and shaking the liquid thoroughly after each addition. The amount of alkali required to neutralise the liquid at this stage should not exceed 2 c.c., and generally less is required. Phenolphthalein is next added to the liquid, and the titration continued until the neutral point is reached with this last indicator. Each c.c. of N/10 alkali required in the second stage of the titration corresponds to 0.0088 grm. of higher alcohols expressed as amyl alcohol. The alkali added when titrating with methyl-orange was formerly supposed to represent mineral acid which distilled, and is still usually not taken into account.

Notes on Above Method.—The brine is best made by saturating water with clean table salt, adding dilute sulphuric acid until the liquid has a distinctly acid reaction, and filtering the solution.

The carbon tetrachloride intended for use in the process must be previously purified by treatment with chromic acid mixture and subsequent distillation over barium carbonate. The carbon tetrachloride recovered at the end of the process may after similar treatment be used again.

The corks used in distilling the spirit must be kept separate from those used during and after the oxidation process. They are liable to absorb amyl alcohol and valeric acid, to prevent which they must all be carefully covered with tinfoil. Rubber bungs should not be used. Schidrowitz and Kaye (*Analyst*, 1905, **30,** 191) recommend a condenser tube ground to fit the neck of the flask used during the eight hours' digestion. They also recommend the use of a Young's "rod-and-disc" apparatus (*Trans. Chem. Soc.*, 1899, **75,** 689) inside the 24 in. condenser-tube.

The steam used for the final distillation must be free from carbon dioxide, since phenolphthalein is to be used as indicator. This condition is easily satisfied by having the steam can or flask briskly boiling some minutes before steam is wanted.

The methyl-orange acidity was formerly attributed to hydrochloric acid, and consequently not taken into account in calculating the higher alcohols, of which the total acidity less the methyl-orange acidity was held to be the measure. It has been pointed out by Schidrowitz and

Kaye (*Analyst*, 1906, **31**, 183) that in the neutralised liquid resulting from the final titration of a carefully conducted determination, only a trace of chlorine can be found, whereas the methyl-orange acidity is almost invariably about 10 % of the total acidity and is, in fact, due to the fatty acids which are not absolutely neutral to methyl-orange. They recommend calculation of the total acidity to amyl alcohol, with the reservation that, if the methyl-orange acidity much exceeds 10 % of the whole, a gravimetric estimation of chlorine is indicated. The preferable plan would be to add to the number of c.c. of barium hydroxide, required in the second (phenolphthalein) stage of the titration, one-ninth or the actual volume required in the first (methyl-orange) stage, whichever is the least, and to repeat the determination if the methyl-orange acidity much exceeds 10 % of the whole. These suggestions are placed in a note and not in the text, as evidence is lacking that they find general adoption. The use of both indicators is advisable in any case, as a check is thus provided on the manner in which the analysis has been carried out.

If an unexpectedly high value for higher alcohols is found and the methyl-orange acidity is normal, there is always a suspicion that some of the ethyl alcohol has remained in the carbon tetrachloride extract and been oxidised to acetic acid. Schidrowitz and Kaye (*Analyst*, 1905, **30**, 193) say that though some ethyl alcohol is certainly extracted and is not entirely washed out, yet this in their experience yields but little acetic acid and is mainly converted into some non-acidic compound. It is, however, easy to determine the mean equivalent of the acids combined with barium hydroxide. To this end, the neutralised aqueous extract is separated from the carbon tetrachloride, evaporated to dryness, dried at 130° C. and weighed. Let the weight be: *a* mg. and *b* the number of c.c. of baryta consumed in determining the total (methyl-orange and phenolphthalein) acidity, supposing the methyl-orange acidity normal. Then the mean equivalent of the acids is given by $10\frac{a}{b} - 67.7$. The equivalent thus determined will, as a rule, indicate that if acetic acid is present, its quantity must be very small. Where the methyl-orange acidity is abnormally high, the abnormal part of it may be calculated to barium chloride first, but in such a case a repetition of the whole process is indicated.

Crampton and Tolman (*J. Amer. Chem. Soc.*, 1908, **30**, 98) recommend the use of an oxidising mixture consisting of 5 grm. of potassium dichromate and 5 c.c. of sulphuric acid made up to 50 c.c.

with water. This quantity is used by them to oxidise the higher alcohols extracted from 50 c.c. of whisky.

Röse-Herzfeld Method.—This method, as slightly modified by Stutzer and K. Windisch, has official sanction in Germany. It depends on the increase in volume of chloroform when shaken up with the spirit under certain rigidly defined conditions. For measuring the increase in volume a special apparatus is supplied by dealers, in whose catalogues it may be found figured and described as a "fusel-oil tube." From a 20 c.c. bulb springs a narrow tube, graduated throughout its length, and this tube is surmounted by a much larger bulb which is provided with a stopper. Several modifications of the tube, differing in the range and fineness of the graduations, are obtainable, but to be of any service they should show 0.02 c.c., and be readable to half this, as the total effective reading may be no more than 0.05 c.c. Alcohol, absolutely free from fusel oil, is required for control experiments; it should be at least twice fractionated over potassium hydroxide and only the middle fractions taken. For use in the test this control alcohol, as well as the spirit under examination, must be freed from carbon dioxide by boiling under a reflux condenser and diluted with great exactness to 30% alcohol by volume; that is to say the sp. gr. must lie between 0.96555 and 0.96560. The apparatus is next charged with 20 c.c. of a mixture of fuming and ordinary concentrated sulphuric acid, rotated so that the whole of the inner surface is wetted by the acid, gradually warmed up and finally kept for an hour in a water-bath not much short of boiling. It is then rinsed with distilled water and dried by a current of dry air. The apparatus is then suspended in a vessel of water at exactly 15°, and anhydrous redistilled chloroform (20 c.c.) poured in down a thistle funnel which extends nearly to the bottom of the apparatus; the object is to fill with chloroform the lower bulb, and the stem up to the lowest graduation mark, or a little above it, without wetting the upper part of the tube. After leaving the chloroform a sufficient length of time to insure its being at exactly 15°, its level is exactly adjusted by withdrawing a fraction of a drop of chloroform by means of a long capillary tube. 100 c.c. of the exactly 30% control alcohol, exactly at 15°, is then introduced, and 1 c.c. of sulphuric acid of sp. gr. 1.268. The apparatus is stoppered, turned upside down so that the contents mix in the large bulb, and shaken vigorously 150 times under water, the temperature of which must remain 15°. The apparatus is then

lifted out of the water and gently inclined so that the chloroform slowly trickles back into the lower bulb; in this way a sharper line of separation is obtained. The apparatus is again suspended in a cylinder of water at 15° and after an hour (not sooner) the reading, *a*, taken where the two layers meet. The whole process is next repeated with the spirit under examination. Let the reading this time be *b*. The German Public Health Department multiplies the number *b-a* by 2.22, and returns the result as parts of fusel oil per 100 c.c. of absolute alcohol in the sample. The method gives results limited in accuracy only by the manner of graduation of the instrument, when applied with every precaution to solutions in pure spirit of the higher alcohols which may occur in fusel oil. Each of the alcohols has the same or nearly the same effect on the chloroform, but commercial spirits are not simply alcoholic solutions of higher alcohols, and the actual reading is the algebraic sum of the readings which would be given by each constituent of the spirit singly. Some of these constituents may cause a contraction of the chloroform column, and Schidrowitz (*J. Soc. Chem. Ind.*, 1902, **21**, 815) has stated that certain samples of whisky actually gave negative results in his hands. The method is described at length in all German text-books, *e. g.*, in Maercker's "*Spiritusfabrication*" (ed. Delbruck, 1903). In the papers of Schidrowitz (*loc. cit.*) and Veley (*J. Soc. Chem. Ind.*, 1906, **25**, 398) those interested will find a fairly complete set of references, but little encouragement to make use of them.

Sulphuric Acid Method.—This method has official sanction in France. Since some brandy shippers allege that it more often confirms their palate judgment than does the Allen-Marquardt test, it will be described here in the first place substantially as its advocates describe it (cf. Girard et Cuniasse, *L'Analyse des Alcools*, 1899). The criticisms to which it has been subjected cannot be incorporated in this description, since they are not helpful, but of such a nature as to absolutely discredit the method.

50 c.c. of the spirit, which by previous dilution or concentration has been brought to 50 % strength, is first boiled under a reflux condenser with some reagent which will fix the aldehydes. 1 grm. of metadiaminobenzene, or 1 c.c. of syrupy phosphoric acid and 1 c.c. of aniline (Mohler, *Ann. Chim. Phys.*, 1891, **23**, 129), is generally used, but Schidrowitz and Kaye prefer calcium phenylhydrazine sulphonate (Hewitt's reagent), and this, if obtainable, is no doubt excellent for the

purpose. A few pieces of pumice are added and the whole boiled for an hour. After cooling, the condenser is rearranged for distillation, and the spirit distilled until 45 c.c. has come over. The distillate is made up to 50 c.c. with distilled water and 10 c.c. transferred to a small dry flask. 10 c.c. of the purest sulphuric acid obtainable are now delivered by a pipette in such a manner that the acid flows down the side of the flask and reaches the bottom without much mixing with the spirit. The contents of the flask are then well shaken and left for an hour on the water-bath. Some workers heat over a naked flame to incipient boiling and then allow to cool, others substitute a brine-bath for the water-bath. After cooling, the colouration developed is compared with that given under the same conditions by a standard solution of isobutyl alcohol in pure 50% ethyl alcohol. This solution is made by dissolving exactly 0.5 grm. of pure isobutyl alcohol in 1000 c.c. of 50% ethyl alcohol. For the purpose of strict comparison 50 c.c. of this solution should be distilled with the chosen de-aldehyding reagent and the first 45 c.c. of the distillate collected and diluted to 50 c.c. 10 c.c. of this solution are treated with 10 c.c. of sulphuric acid and heated exactly like the spirit under examination. The resulting liquid constitutes the colour standard. If the tints of the standard and of the assay liquid are identical, the 50 % spirit under examination may be returned as containing 0.083 % of higher alcohols, since average fusel oil is said to develop only 0.6 of the colour given by isobutyl alcohol. Results are more conveniently returned in mg. per 100 c.c. of absolute alcohol. thus a sample exactly matching the standard would be returned as containing 167 mg. per 100 c.c. of absolute alcohol. If, as is usual, the tints of the standard and assay liquid differ, it is necessary to compare them accurately. Numerous special colorimeters have been devised with the object of facilitating this comparison, but it is to be noted that the colour does not vary directly with the content of higher alcohols, and that something more than a rule-of-three sum is required in calculating the results. Suppose, for example, that a layer of the assay liquid has the same intensity of colour as a layer of the standard only half its depth, the number to be returned is not 83, but 116 mg. of higher alcohols per 100 c.c. of absolute alcohol. Girard and Cuniasse (loc. cit.) give a curve and table connecting "apparent" and "real" content of isobutyl alcohol, but as this is only a secondary constituent of fusel oil, the following table is perhaps more useful.

Ratio of intensity of colour of assay liquid to that of standard.	Mg. of higher alcohols per 100 c c. of absolute alcohol in sample.
0.1	53
0.2	77
0.3	92
0.4	105
0.5	116
0.6	127
0.8	147
1.0	167
1.2	184
1.4	201
1.6	218
1.8	235
2 0	252

The above table is calculated from the curve of Girard and Cuniasse on the assumption that "average" fusel oil produces only 0.6 as much colour as its own weight of isobutyl alcohol under the conditions of the test. The last figure of each of the numbers in the right-hand column has no justification, but the writer has not yet met with any chemist who is content with a 0 in this place. "Average" fusel oil is but a figment of the imagination. The proportions of the higher alcohols present differ with the raw material and manner of distillation of the spirit, and each one has its own capacity, greater or less, for producing colour in this test. Experiment shows that the numbers obtained in this test bear no constant relation to those obtained by the Allen-Marquardt method, which, whatever its imperfections, is based on scientific principles. The most serious criticism to which the test has yet been subjected, however, is that of Veley (*J. Soc. Chem. Ind.*, 1906, 25, 400), who found that isobutyl alcohol itself, if carefully purified, gives no colouration with pure sulphuric acid. The reason for devoting so much space to so unsatisfactory a test has been already given, and is sufficient. Even Veley says the test is capable of giving valuable information. No one will refuse a chemist the right to make any test which aids him in forming a judgment regarding a sample, but if the number obtained by the sulphuric-acid test is returned in a certificate as a measure of the higher alcohols, it is reasonable to require the addition of the words "colorimetric method," since the colour may be and

probably is the measure of something else and not at all of the higher alcohols.

It has already been said that the Allen-Marquardt method is the only one which can be generally recommended. No method for the estimation of such a variety of substances as is included under the heading of "fusel oil" can be entirely satisfactory, and isopropyl alcohol is theoretically not estimated by the Allen-Marquardt method, since on oxidation it yields acetone and no acid. Jenks and Bedford (*J. Soc. Chem. Ind.*, 1907, **26**, 123) find that the Allen-Marquardt method greatly underestimates every constituent of fusel oil except amyl alcohol, and they have devised a method which they allege enables them to differentiate between amyl alcohols on the one hand and butyl and propyl alcohols on the other, but little éxperience has yet been gained with the method, and so far as the writer knows it is used only by its authors.

Acids and Esters.—100 c.c. of the spirit is distilled until only about 10 c.c. remain; distillation is then continued by passing in steam, free from carbon dioxide, as in the Allen-Marquardt process for the estimation of higher alcohols. The bulk of the distillate should be about 150 c.c. and the residue left in the flask not much more than 5 c.c. This residue may be diluted with water and the fixed acid determined by titration with N/10 alkali, using phenolphthalein as indicator, and the result calculated in terms of tartaric acid. Only spirits which have been stored long in wood contain any appreciable amount of fixed acid.

The distillate contained in a Jena flask is exactly neutralised with sodium hydroxide, using phenolphthalein as indicator, and the volatile acid calculated as acetic acid, though higher acids are certainly present in some spirits. A further 10 c.c. of N/10 alkali is now added and the whole boiled under a reflux condenser for half an hour. After cooling 10 c.c. of N/10 acid is added and then N/10 alkali to exact neutralization. The amount of N/10 alkali required in this last titration is calculated in terms of ethyl acetate, though more complex esters are no doubt generally present.

Allen preferred to remove aldehydes before proceeding to the estimation of esters. Though this is desirable on theoretical grounds, in practice the error involved by neglecting the action of the aldehydes on the standard alkali is very small, whereas the use of any of the dealdehyding reagents suggested may introduce errors of unknown

magnitude. Hewitt's reagent (sodium or calcium phenylhydra-zine-*p*-sulphonate) is the least objectionable of these reagents, but Hewitt himself (*Analyst*, 1905, 30, 153) does not recommend its use in this connection.

Bulletin 107, (U.S.Dept. of Agric) recommends that after distilling the alcohol over sodium hydroxide, 3 grm. of metaphenylenediamine should be added and the mixture allowed to stand for several days at room temperatures or boiled in a reflux condenser for several hours, then distilled slowly rejecting the first 100 c. c. and the last 200 c.c.

Furfural.—In a colourless spirit this is easily estimated by comparing the tint produced in the liquid by the addition of aniline acetate with that produced in a standard solution of furfural in pure 50% alcohol. The alcohol used for preparing and diluting the control solution must be free from aldehyde. It is digested with potassium hydroxide and fractionated, and only the portion boiling between 78° and 80° collected. If this gives any colouration with aniline acetate, the treatment should be repeated or recourse may be had to any of the de-aldehyding reagents already mentioned. In the reviser's experience the glacial acetic acid supplied to analytical chemists never contains furfural or even traces of those bodies, present in commercial acid, which develop a yellow colour with aniline. It is convenient to boil for a few minutes equal bulks of aniline, acetic acid and water. The mixture when cool constitutes the reagent, and the boiling effectually destroys any furfural which might be present in the acid. The most convenient strength for the control liquid is 0.05 grm. furfural per 1000 c.c. of 50 per cent. alcohol, and it is of course made by diluting a stronger solution. Since the colouration is in some measure dependent on the alcoholic strength of the liquid, it is advisable, when the spirit under examination differs much from 50% strength, to dilute the control liquid with water or pure alcohol until its alcoholic content approximates that of the sample. To 20 c.c. of the spirit and 20 c.c. of the control solution, each contained in Nesslser glasses, 1 c.c. of the aniline-acetate solution is added, and after ten minutes the tints compared. Some of the darker solution is now withdrawn until, on looking down the tubes, the tints appear identical. If the control liquid was diluted to adjust its alcoholic content, this must not be overlooked in the calculation, which is otherwise similar to that applied in nesslerising.

When, as is usual, the spirit has considerable colour, this must

in some manner be removed. Hewitt (*J. Soc. Chem. Ind.*, 1902, 21, 98) recommends distilling nearly to the last drop, adding pure dilute alcohol to the distilling flask, and again distilling nearly to the last drop, and so on three or four times. The united distillates are then made up to some definite volume. Schidrowitz (*J. Soc. Chem. Ind.*, 1902, 21, 816) strongly criticises this procedure mainly on the ground that furfural may be formed during the distillation. He prefers to decolourise as far as possible with lead acetate, and then to add the aniline reagent to the liquid under examination and to the control. If the shades (not the intensity of colour) differ, dilute tincture of galls is added to the control until they match. The tincture of galls is added after, and not before, the reagent because it is intended to neutralise not only the tint remaining in the spirit after treatment with lead acetate, but also the yellow colour which certain aldehydic bodies give with aniline. To 20 c.c. of the sample, Schidrowitz adds a few drops of basic lead acetate solution, shakes, adds enough saturated potassium-sulphate solution to precipitate the excess of lead, filters and proceeds as above described.

Aldehydes other than furfural. Many methods for the estimation of aldehydes in potable spirits have been described. The only one in common use, however, is a colorimetric estimation by means of Schiff's reagent. The usual formula for the reagent is:

0.15 grm. of fuchsin in 150 c.c. of water,
100 c.c. of sodium hydrogen sulphite solution (sp. gr. 1.36),
10 c.c. of concentrated sulphuric acid.

As the presence of much mineral acid greatly reduces the sensibility of the reagent, the following modification is recommended:

0.2 grm. rosaniline base is dissolved in 20 c.c. of a cold saturated solution of sulphurous acid; if the colour is not discharged after 24 hours, a further 10 c.c. of sulphurous acid is added; after a further 24 hours the colour will usually be discharged, but if not, more sulphurous acid is added and the solution when finally decolourised is diluted to 200 c.c. with water. Some samples of rosaniline yield yellowish-brown solutions which cannot be entirely bleached, but when diluted to 200 c.c., the colour, even of bad samples, is seldom of serious account.

A control solution of acetaldehyde in pure 50 % alcohol is required. A convenient strength is 0.2 grm. acetaldehyde per 1000 c. c. The alcohol must be freed from aldehyde similarly to that used in pre-

paring the furfural control. It is convenient to prepare a stock which reacts neither with Schiff's reagent nor with aniline.

Bulletin 107, U. S. Dept. of Agriculture, gives the following as a provisional method for preparing a standard aldehyde solution. Grind aldehyde ammonia in a mortar with ether and decant the ether, repeating this operation several times; then dry the purified material, first in a current of air and then in vacuum over sulphuric acid. Dissolve 1.386 grm. of this substance in 50 c.c. of 95% alcohol, purified from aldehyde; to this solution add 22.7 c.c. of N/1 sulphuric acid, made with alcohol instead of water, make up to 100 c.c. and add 0.8 c.c. to compensate for the volume of the ammonium sulphate precipitate. Let the liquid stand overnight and then filter. The solution contains 1 grm. of aldehyde in 100 c.c. and keeps well.

The most convenient standard is prepared by adding 2 c.c. of the above solution to 100 c.c. of 50% (by volume) of alcohol; 1 c.c. of this dilute solution contains 0.0002 grm. aldehyde. This dilute solution does not keep.

The test is carried out by adding to 20 c.c. of the liquid under examination and to 20 c.c. of the control solution, 5 c.c. of the reagent, and comparing the tints produced after 20 minutes. Portions of the darker are withdrawn until, on looking down the tubes, the tints appear equal. Dubosc's or other colorimeter is used by those who make many of these determinations, but the chemist in general practice may use Nessler glasses, and calculate on the assumption, not quite true, that the intensity of colour is proportional to the amount of aldehyde present. The influence of furfural may be neglected, since it gives a very faint colouration with Schiff's reagent, compared with that given by acetaldehyde. It is desirable that the solution under examination and the control solution should be of approximately the same alcoholic strength, and this is effected by adding to one or other of them water or pure alcohol.

Highly coloured spirits are best treated by Schidrowitz's method, described under Furfural. The spirit is decolourised as far as possible with basic lead acetate, the excess of the latter removed by the addition of potassium sulphate, and the liquid filtered. The control is then coloured with tincture of galls until it exactly matches the sample, Schiff's reagent added to both control and assay liquid and the comparison made after 20 minutes.

Non-volatile Residue.—This is sometimes of importance. When

freshly distilled, spirits contain no trace of non-volatile matter. When kept in casks they take up more or less fixed matter, but the amount rarely exceeds 100 grains per gallon. The fixed matter may include, among other substances, tannin, colouring matter, sulphates and traces of sugar. The proportion of non-volatile matter in spirits is ascertained by evaporating 50 or 100 c.c. to dryness on a water-bath. Some indication of its nature may be obtained by tasting the residue. On ignition in the air, any zinc, lead, or copper present in the spirit will be left as an oxide. Very sensible traces of these metals may be present accidentally, and there is good evidence that their salts were in the past occasionally used as adulterants. Occasionally, clarifying materials containing lead acetate have been employed. Alum was also used occasionally. The reaction of the ignited residue should be observed, as, if alkaline, an alkaline carbonate, acetate, tartrate, etc., must have been present.

Sulphates will be detected on adding barium chloride to the diluted spirit. Free sulphuric acid has been met with in whisky, and is said to have been used formerly for adulterating gin. This is extremely improbable. The presence of free sulphuric acid may be detected by the methods used for examining vinegar for mineral acids.

Tannin is often present in brandy, being chiefly extracted from the casks used for storing. Sometimes it is purposely added in the form of tincture of galls or oak-bark. It may be detected by the darkening produced on adding ferric chloride to the spirit, and any reaction thus obtained may be confirmed by boiling off the alcohol from another portion of the spirit and adding solution of gelatin to the residual liquid, when a precipitate will be produced if tannin be present.

A few analyses of spirits are given here, not to serve as "types" nor to prove the folly of referring spirits to types, but to give some idea of the results to be expected. Girard and Cuniasse, at the end of their book and elsewhere, have published a large number of spirit analyses; the most interesting of their numbers are those which relate to brandy, but they give several examples of French industrial alcohol. Probably the whiskies selected by Schidrowitz (*J. Soc. Chem. Ind.*, 1902, **21**, 818) are more typical of the spirit consumed in Great Britiain than are the whiskies on which continental chemists report from time to time. Vasey (*Analysis of Potable Spirits*, London, 1904) gives a number of analyses, some from continental sources but many original, while in König's "*Chemie der menschlichen Nahrungs- und Genussmit-*

tel" there are many more.. Reference to Vasey may lead the analyst to suppose that the judgment of spirits is comparatively simple, and Girard and Cuniasse appear to base confident judgments on analytical data, but some of their own selected analyses invalidate the standards they suggest. Both Vasey and Girard and Cuniasse, it is true, assume that the analyst will call on his palate to aid him in his judgment, but they may fairly be quoted as representing the school which believes most strongly in the ability of the chemist, *qua* chemist, to decide whether spirits are "genuine" or otherwise. As a corrective to too great confidence in numbers, the analyst may be referred to a communication by Windisch (*Zeit. Unters. Nahrungs- und Genussm.*, 1904, 8, 465) and to a paper on "Brandy," by Hehner (*Analyst*, 1905, 30, 36).

RESULTS OF ANALYSES OF POTABLE SPIRITS.

	Alcohol per cent. by vol.	Milligrams per 100 c.c. of absolute alcohol.				
		Higher alcohols.	Esters.	Acid.	Furfural.	Aldehydes excl. furfural.
1. Brandy, genuine grape, 2 years.	64.4	253	136	77	1.3	19
2. Brandy, genuine grape, 16 years	61.1	95	81	59	1.0	24
3. Brandy, genuine grape, 35 years	47.5	345	133	202	1.2	48
4. Brandy, admittedly blended with patent spirit.	50.0	60	67	58	0.9	18
5. "Brandy," admittedly flavoured patent spirit (no grape)..	50.0	18	32	19	0.2	6
6. Sold as "cognac".	64.0	Nil	14	11	0.3	2
7. Whisky, malt, new.	62.8	189	70	16	4.4	11
8. Whisky, malt, 4 years	60.5	217	95	55	3.0	23
9. Whisky, grain, new	61.5	76	48	Nil	Nil	5
10 Whisky, grain, 4 years.	59.7	77	77	11	Nil	11
11. Rum, Jamaica, genuine.	69.5	94	440	176	2.9	22
12. Rum admittedly blended with patent spirit.	36.0	114	83	127	0.9	11
13. Sold as "rum".	55.0	8	45	65	0.6	6
14. Gin.		45	37	Nil	Nil	2
15. Highly rectified spirit.		3	3	3	Nil	0.1

Brandy is usually defined as a spirituous liquid, distilled from wine and matured by age. The best, that is the most palatable and valuable brandies, are no doubt produced in this way, but it is difficult, if not impossible, for a chemist to decide with certainty whether a particular sample of "brandy" is properly so described. It is true that on the average brandies contain 80 to 100 mg. of esters per 100 c.c. of absolute alcohol, and that some, including some of the finest, contain much more, but some genuine wine brandies contain

less than half this amount, and there is nothing to prevent a distiller from obtaining pure alcohol from wine except the consideration that pure alcohol is flavourless and not saleable at the price obtained for less pure distillates. On the other hand, except as regards the all-important flavour, there is no difficulty in producing a spirit, innocent of grapes, but complying with any of the standards which have been laid down. A little rum, with 400 mg. esters per 100 c.c. will supply the necessary esters to a large bulk of silent spirit and so on with other constituents. The sum of the higher alcohols, esters, acid, aldehydes and furfural is usually over 300 mg. per 100 c.c. of absolute alcohol, but it is generally agreed now that no rules can be laid down for this total which was formerly spoken of as the "coefficient of impurities."

Nor is it easy to decide on the age of brandy or other spirit. Some oxidation with formation of aldehyde and acid is to be expected and the increased acid determines some further esterification, but as spirits start with such widely different compositions it is possible for one 20-year-old brandy to be indistinguishable analytically from a new brandy from another still. No. 2 is a case in point. The low ester number, which would cause some to doubt the age of this sample, is no doubt due to the manner of storage which was such that comparatively little oxidation took place with consequent small increase of the acidity on which the degree of esterification probably depends. It is highly improbable that any distiller of genuine grape brandy would deliberately refine his product so as to get an article like No. 6, but the figures given do not prove that the spirit was other than brandy as defined by the British Pharmacopœia.

Whisky.—A Royal Commission is engaged in hearing evidence as to what whisky should be. Like other potable spirits it is more or less aqueous alcohol, containing a small proportion of other matters which give it the characteristic flavour associated with the name; flavours, perhaps, would be more correct, since several types of whisky are distinguished by makers and drinkers of the beverage. It may be derived exclusively from malt and distilled in the comparatively simple pot still, or mainly from raw grain and distilled in a patent still which is capable of bringing about very complete rectification. The whisky distiller does not work his patent still so as to bring about the maximum degree of rectification of which it is capable, and in pot-still distillation about two-thirds of the total volatile impurities are eliminated with the pot ale and spent lees, so that the difference between pot

still and patent spirits is not necessarily so great as is sometimes supposed. It is incorrect to speak, as was done in the last edition of this work, of the pot still as an apparatus in which little or no fractionation occurs, but equally incorrect to lay stress on the temporary separation of the distillate into three fractions as do Schidrowitz and Kaye (*J. Inst. Brew.*, 1906, **12**, 496). Of these fractions the first and last are added to the next charge to recover the alcohol contained in them, and the only certain measure of impurities eliminated is the amount contained in the pot ale and spent lees, which with whisky are the sole ultimate products of pot-still distillation. Schidrowitz and Kaye have shown that the esters of the foreshots and feints may be partially hydrolysed on repeated distillation, and that aldehyde may be oxidised to the much less volatile acetic acid is probable, but their figures, based on a single run, scarcely justify their conclusion that only 10 % of the total impurities find their way into pot-still whisky; in fact, they may equally well be made to support an estimate of 30 %.

A large proportion of the whisky sold, and approved by its purchasers, is a blend of grain whisky containing notably less impurities than Nos. 9 and 10 with a pot-still whisky containing notably more impurities than Nos. 7 and 8.

AMERICAN WHISKY is the subject of a special paper (*J. Amer. Chem. Soc.*, 1908, **30**, 98) by Crampton and Tolman, who show that its composition may range within wide limits. Thirty whiskies were examined and, what is more important, were preserved in bonded warehouses in barrels, which were opened once a year for 8 years and a sample from each withdrawn and analysed. No such thorough investigation into the effects of long storage in wood has been made before, and if it is said that no new information has been brought to light, the answer is that this investigation transforms into facts what were previously no more than reasonable hypotheses. The result of this investigation is to establish the following facts Water passes more easily than alcohol through the pores of the wood, with the result that the alcoholic strength of spirits stored in wood increases about 1% per annum. The increase in the percentage of higher alcohols with age is entirely explained by the diffusion of water and ethyl alcohol through the pores of the wood, which appears to be practically impervious to the higher alcohols. The other "impurities" do actually increase in amount, the increase being comparatively rapid during the first 3 or 4 years and after that proceeding very

slowly. The work of Crampton and Tolman also establishes the fact that the source of furfural in whisky is two-fold; it may be derived from the grain of the mash or from the charred wood of the barrel. The aroma and flavor of the whisky are derived from the charred interior of the barrel.

Rum, especially Jamaica rum, is usually characterised by its high content of esters and volatile acid and by its flavour.

Gin is made by flavouring highly rectified spirit with oil of juniper berries or other substances, with or without the addition of sugar. Previous to sale the gin is broken down considerably by addition of water.

YEAST.

By EMIL SCHLIGHTING.

Yeast is an organized ferment, belonging to a class of fungi grouped botanically as "budding fungi" and generally characterized by their faculty of causing alcoholic fermentation in a saccharine solution and by their mode of propagation by "budding," although at times propagation by fission has been observed.

The yeast fungi constitute the genus "Saccharomyces," which is again subdivided into many species. The Saccharomycetes, or yeast fungi having the distinctive faculty of forming endospores, are the only and most important ones for the fermentation industry, while all other yeasts and many Saccharomycetes are of no value to the industry and arts; in fact, some of these are frequently detrimental. The yeast plant is abundantly distributed thoughout the vegetable kingdom and in the air.

Physical Appearance.—Observed in the distillery and brewery, it forms a pale yellowish-white frothy mass with a peculiar ethereal odor and generally bitter taste. The brewer distinguishes between *top* and *bottom* fermenting yeast; the former acts at temperatures of 18° to 25° and appears at the surface of the liquid while the latter ferments at temperatures from 4° to 10° and settles at the bottom of the fermenting liquid.

Microscopical Structure.—Examined under the microscope, yeast appears in the form of many small cells of 7 to 10µ in diameter.

They are seen as either single cells or colonies; their shape differs with the various species from a round to oblong, sometimes elliptical form, but even this variation of form occurs in the same species, so that, according to Hansen, a grouping or differentiation of species by this means alone becomes almost impossible. The yeast cell consists of a colourless cell wall with equally colourless cell contents; the latter consisting of

    a. Protoplasm,
    b. Nucleus,
    c. Vacuoles, and
    d. Some other granular enclosures of various description.

The Cell Wall.—This is very thin, generally 0.5 to 1.0μ; but this thickness is observed, according to Will, mostly in yeasts which have been accustomed to ferment very concentrated worts. The cell wall is generally supposed to consist of two or more layers; it does not turn blue with a mixture of iodine and sulphuric acid, which colours the membrane a brownish-yellow like other fungi, proving that the cellulose of yeast is not closely related to starch. The several layers of the cell wall can be made visible, according to Will and Casagrandi, by a protracted treatment with 1 % chromic acid or with concentrated hydrochloric acid. The outer layers of the thicker membranes are sometimes shed by the cells, as has been observed by Lindner and Will.

Chemical Nature of Cell Wall.—According to investigation, *true* cellulose does not occur in the cell wall of yeast as it resists the solvent action of ammoniacal cupric hydroxide (Schweitzer's reagent) and it is not coloured blue by zinc iodochloride. After treatment with 4 % hydrochloric acid only, the cell wall is stained by Hanstein's aniline violet (according to Becker); this is disputed by Casagrandi.

Lindner found that as an exception, the membrane of spores of *Schizosaccharomyces octosporus* is coloured blue by a solution of iodine in potassium iodide. The yeast cell membrane will be dissolved by concentrated sulphuric and chromic acids, but not by any dilute acids. Alkalies and Schultze's macerating fluid have a tendency to clear up the cell wall.

According to Will and Casagrandi, the two layers of thick membrane differ in their behaviour towards chromic acid, the inner one being dissolved somewhat quicker than the outer; Casagrandi therefore assumes that the cell wall, owing to its resistance towards staining agents and solvents, consists mostly of pectose or a substance closely resembling pectins.

Salkowski succeeded in isolating from yeast by means of potassium hydroxide two cellulose-resembling bodies, which upon hydrolysis produced dextrose or glucose and mannose. Hansen later observed a certain relation between the cell wall and the so-called gelatinous network, which is formed under certain conditions. He found that the membrane excreted a mucilage which on drying formed the peculiar network. This occurs in spore cultures on gypsum blocks and, according to Jörgensen, also if the yeast dries between blotting-paper. Washing removes the gelatinous network, but it is reproduced, provided the washing is not carried too far. The network is precipi-
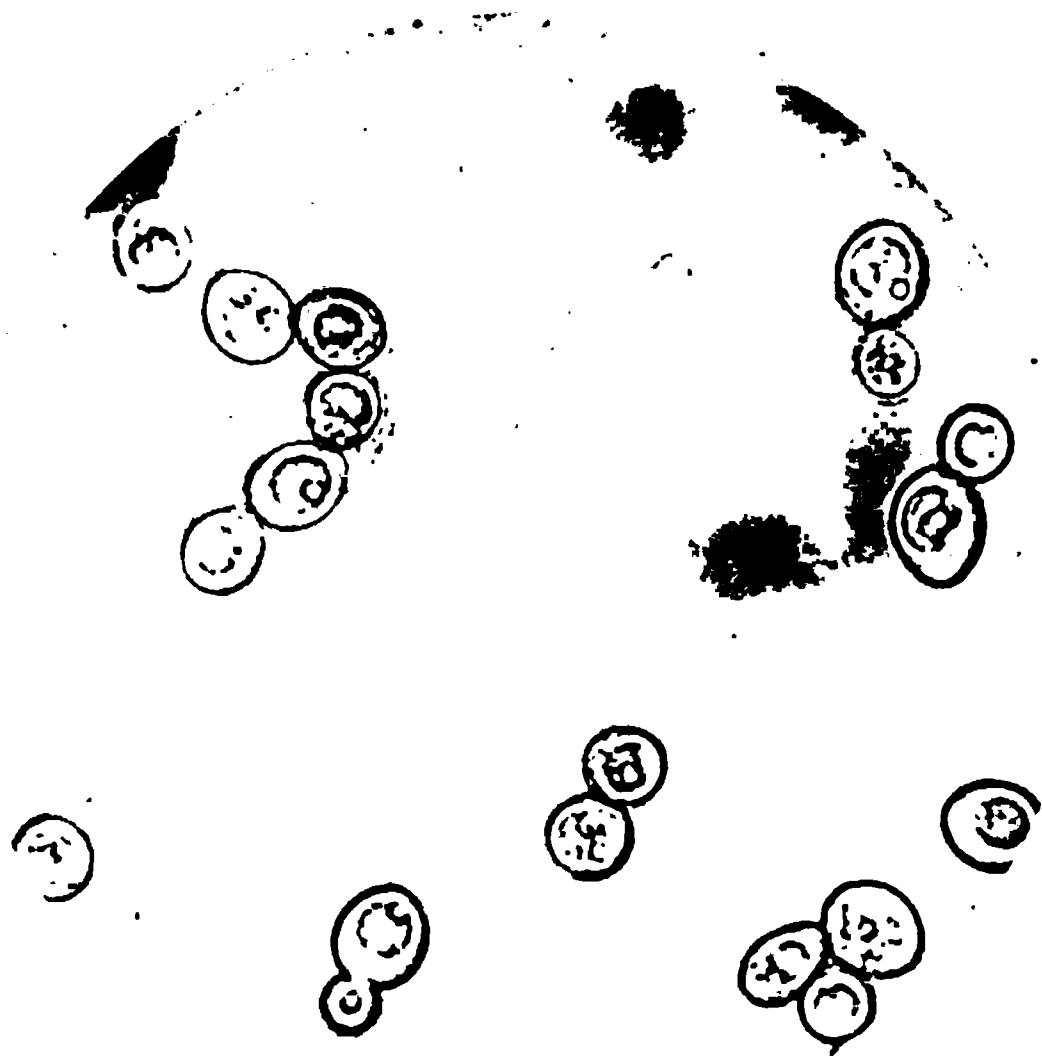
FIG. 57.—Ale Yeast.   Top fermenting.

FIG. 58.—Lager Yeast.   Bottom fermenting.

tated by a 5% borax solution similar to plant gelatins; this is utilized in practice for facilitating the settling and pressing of yeast by the addition of borax. Will considers the formation of this network due to a gelatinization of the cell membrane, the protein content also taking an active part. The exact constitution and formation of this network under certain conditions and in various yeasts have not been established, but former investigators, *i. e.*, Nägeli and Pasteur, have attributed to yeast the faculty of separating and excreting protein bodies or peptones.

Cell Contents.—The cell nucleus is difficult to distinguish in the living cell. It may be made visible by staining. It is generally spherical, sometimes disc-shaped. Its diameter is about 1/3 of the whole cell.

According to investigations of Janssens and Leblanc, there exists only one nucleus in each cell. Dangeard, Janssens and Wager assert that it encloses a nucleolus or granular body, possessing a membrane. The intermediate space consists of a fine network of granular protoplasm. At the end of fermentation, at a stage of exhaustion, vacuoles appear filled with a fluid of unknown composition, differing from protoplasm in lower refraction. They sometimes occupy the largest portion of the cell and also contain crystalline enclosures. Frequently they have been observed to contain very small granular bodies which are in constant motion (Brownian movement). Küster considers them decomposition products of protoplasm of a semi-liquid consistency which eagerly absorb stains.

Strange refractive bodies are often seen in the protoplasm, appearing generally at the end of fermentation; these were formerly considered as *oil drops;* they are now called *granules;* their number and size differ considerably in the various cells; they are at times round and then angular. According to Will, their membrane consists of proteid matter, with a similarly constituted interior network; their contents are of a fatty nature; as it may be removed by fat-dissolving reagents (ether, chloroform, alkalies, alcohol, petroleum spirit). The proteid cell wall is dissolved by concentrated sulphuric acid, the oily drops flow together, and color first green, then bluish-green and finally black. Absolute alcohol added to yeast causes the cells to shrink, and they are soon killed. Dead cells are generally distinguished from live ones by their greater absorbing faculty and ease of staining

Chemical Composition of Yeast.—The percentage composition

of yeast shows only very slight differences in the analyses of top and bottom yeasts. The nitrogen is usually somewhat higher in top yeast, but generally the composition will depend on the nutrition. According to Mitcherlich, Schlossberger, Dumas, Wagner and Liebig, the ash-free dry substance of yeast has the following composition:

	Carbon	Hydrogen	Nitrogen
Top Yeast,	48.64	6.76	11.46
Bottom Yeast,	44.99	6.72	8.73

Older yeast, according to Schlossberger, is generally somewhat poorer in nitrogen, owing to decomposition of its cell contents.

The moisture ranges from 75 and 83 %.

The sulphur ranges from 0.39 and 0.69 %. (Liebig).

Ash of Yeast.—Investigators differ considerably in their results regarding the ash content. This is stated for top yeast to be from 2.5 to 11.5%; for bottom yeast from 3.5 to 10.1%. yeast. Too much reliance, however, cannot be placed upon these figures, as the original materials for analysis were not uniform or have not been stated. The ash is stated to consist of phosphates, sulphates, silicates, chlorides and potassium, sodium, magnesium, and calcium; potassium phosphate constituting the largest proportion.

Nitrogenous Constituents.—Mostly proteins; Schlossberger extracted with potassium hydroxide a substance containing 13.9% nitrogen, Mulder obtained with dilute acetic acid a substance with 16% nitrogen, and Nägeli and Loew found in a bottom yeast with 8% nitrogen as follows: Albumin, 36%; glutin-casein, 9%; peptones, 2%. The nuclein bodies, forming the main constituents of the cell nucleus have been studied closely and were isolated by Kossel, and their presence proved by Hoppe-Seyler. Stutzer found in a beer yeast having 8.65% nitrogen, 2.26% present as nuclein.

The protein-like substance formed by the action of dilute alkalies upon nuclein resists the action of pepsin and trypsin. Other protein bodies not yet clearly defined are thought to form a gelatinous network around the cells; these are also partly transferred to the beer and aid in the retaining of larger amounts of carbonic dioxide. Reichard maintains that these gelatinous bodies are indispensable for the production of a fine, creamy foam in beer.

Fat.—The fat content fluctuates with the nutrition of the yeast. Nägeli and Loew state it to be about 5 %. It consists, according to

Darexy and Gérard, mainly of stearic and palmitic acids and a little butyric acid, partly as glycerides, partly free. It serves as a reserve food material. Lecithin and cholesterol have also been isolated from yeast by Hoppe-Seyler.

Carbohydrates of Yeast.—

The following have been isolated:

1. Glycogen.
2. Yeast-pectose.
3. Yeast-cellulose.

Yeast-glycogen was first obtained by Cremer (1894), who proved it to be identical with the glycogen of the liver. The dry substance of yeast contains between 31 and 32% of glycogen; its percentage may be increased by suitable nourishment. Contrary to the claim of Laurent, glycogen cannot be absorbed and assimilated from nutrient solution by yeast. Henneberg asserts that the various types of yeast can be distinguished by the extent of glycogen formation.

According to investigations made by Cremer, and later confirmed by Buchner and Rapp, yeast also contains an enzyme capable of converting glycogen into a glucose. "Yeast gum" or yeast pectinous substances have been isolated by several investigators.

Yeast cellulose or substances resembling cellulose are contained in the membrane of the cell. This cellulose behaves differently from ordinary pure cellulose; is insoluble in ammoniacal cupric hydroxide and gives none of the usual cellulose reactions. Salkowski obtained by extraction with a 3% potassium hydroxide a substance resembling cellulose, having a constitution of $C_6H_{10}O_5$; boiled in water it was split into a soluble substance giving a red color with iodine, and another insoluble jelly-like substance. The former, so-called erthyrocellulose, gave on hydrolysis only dextrose, while the latter resulted into achroocellulose and a small amount of mannose.

Payen states cellulose to be present in dry yeast up to 29.4%. Liebig and Pasteur found only 16 to 18%.

Tannin.—Jörgensen claims the presence of tannin in yeast during the first stages of fermentation, but Naumann and Will were not able to find it.

Mineral Constituents of Ash.—There are many analytical data by different authorities regarding the ash constituents of yeast and they generally are found to range between the following limits:

Potassa (K$_2$O)	Soda (Na$_2$O)	Magnesia (MgO)	Lime (CaO)
23.3 to 39.5	0.5 to 2.5	4.1 to 6.5	1.0 to 7.6
Phosphoric Acid (P$_2$O$_5$)	Sulphuric Acid (SO$_4$)	Silica (SiO$_2$)	Chlorine
44.8 to 59.4	0.3 to 6.4	0.9 to 1.9	0.03 to 0.1

Vitality of Yeast.—According to Hansen, yeasts retain their vitality longest in a 10% sucrose solution. Of 44 species after 20 years' observation only 3 varieties died in this solution. They die quicker in wort, also in water, but generally keep for a period of several months to years. Drying in a very finely divided state kills yeast after a few days; some varieties may live for several months; spores are more resistant. Dried on filter paper or cotton, yeast may retain its vitality for at least one year; spores two or three years. Will made thorough and successful experiments by drying yeast with powdered wood charcoal; the yeast was still alive after 10 years.

Heat.—Moist heat is detrimental to yeast, and kills it between 50 and 60°; spores are more resistant. In a wine with 6.4% alcohol the yeast cells were killed after heating at 45° for 2 hours. Cooling to —130° and freezing for months is not detrimental to the yeast cells.

Light.—Diffused daylight and electric arc light retard the budding; sunlight kills the cells. It is not known whether yeasts also participate in the detrimental action of sunlight upon the taste and odor of beer.

Characterisation of Saccharomycetes.—If brought into saccharine fermentable solutions, yeast will form a sediment which increases with the period of fermentation; in breweries and distilleries this consists mostly of round or oval cells. Such yeasts are classed as the *Saccharomyces cerevisiæ* type.

Wine yeasts and some other types are elliptical, and are classed, according to Rees, as the Ellipsoideus type. A third type, called *Pastorianus*, is characterized by its elongated sausage-shaped form. These latter, Pastorianus yeasts, are generally detrimental to the fermentation process and are considered as disease ferments.

Although the cells of one type are not always strictly uniform, there exist for the most part a larger number of characteristic cells enabling positive identification.

Spore Formation.—Besides vegetative propagation by budding, the Saccharomycetes cells also form endospores, the cell being transformed into an ascus. Hansen has used the different spore formation and its properties to differentiate between the various types of culture and wild yeasts. The spores of culture yeasts appear to be empty,

while the spores of wild yeast are strongly refractive. These phenomena are utilized in the analysis of brewery yeast.

Enzymes.—The yeast-cell contains many enzymes distinct from each other in their respective action. The kind and number of enzymes in different types differ materially and may be considered as one of the safest and most constant factors of identification and differentiation.

Some of these enzymes have the faculty of diffusing through the cell membrane; others are partly incapable of diffusion and are then utilized for assimilation and disassimilation within the cell. Hahn proposes for these enzymes the name "endoenzymes."

A synthetic action of yeast enzymes has only been proved for "yeast glucase" by Croft Hill and Emmerling, the final product from dextrose being maltose, according to Croft Hill, and isomaltose, according to Emmerling.

The yeast enzymes may be grouped as follows:

1. *Hydrolysing enzymes:*
   a. *Sugar splitting:*
      Invertase, Maltase, Lactase, Melibiase, Raffinase, Trehalase, Diastase and a glycogen-splitting enzyme.
   b. *Proteolytic.*—Endotryptase.
   c. *Coagulating.*—Rennet.
2. *Oxidising:* Oxydase, Catalase.
3. *Reducing enzymes.*
4. *Fermenting enzymes:* zymase.

Invertase splits sucrose into dextrose and lævulose; raffinose is broken up into lævuose and melibiose (Bau). Invertase was first isolated by Berthelot. It occurs in brewery and other culture yeasts as well as in most wild yeasts, is easily soluble in water, thereby differing from other sugar-splitting enzymes, acts only in acid solution and is not affected by drying for one hour at 140 to 150°.

Maltase changes maltose into dextrose; is difficultly soluble in water and can only be extracted from crushed and ground cells by leaching; it occurs in most yeast types; optimum temperature (Lindner and Kroeber), 40°. It is destroyed, according to Beyerinck, at 50 to 55°.

Melibiase.—This splits melibiose into dextrose and galactose; it is soluble in water and has been extracted from bottom fermenting yeasts by leaching dried cells with water. It occurs also in some of the top fermenting yeast types (Lindner).

Raffinase splits raffinose, but not sucrose. It occurs in several yeasts.

Lactase splits lactose into d-galactose and dextrose and occurs in only a few saccharomycetes, never in brewery culture yeast. It has been found in Kefir organisms; it does not diffuse or penetrate the cell wall.

Trehalase, splits trehalose, not diffusing. E. Fischer proved its presence in the Frohberg type, and considers it identical with diastase; Effront does not agree with this view.

Glycogen-splitting Enzyme.—This was found in the yeast juice obtained by E. Buchner under high pressure. It ferments glycogen which is *not* accomplished by the yeast proper. It probably plays an important part in the "so-called" *self-fermentation or auto-digestion.* Wroblewski considers it identical with diastase.

Diastase.—Starch is also attacked (Wroblewski) by yeast juice in a small degree, while the yeast proper has no action upon the same. Lately yeasts have been discovered capable of fermenting dextrins.

Proteolytic Enzyme.—The presence of this enzyme was proved by Will, Wehmer, and Beyerinck. Beyerinck considers it similar to trypsin, as proteolysis is stronger in alkaline than in acid gelatin. Hahn, however, claims it must act in an acid solution; its optimum action occurs in 0.2% solution of hydrochloric acid (similar to pepsin). Yeast juice proteins lose their power of coagulation after 10 to 14 days' auto-digestion. The products are tyrosin, leucin, xanthin bodies, passive albumoses, but no peptones.

Hahn and Geret have established the following properties of yeast-endotryptase; it is precipitated from yeast juice by alcohol; cannot be separated from invertase; gives no reaction with Millon's reagent. Optimum temperature, 40 to 45°, destroyed at 60°; retains its efficiency in yeast juice 9 to 15 days at 37°. Endotryptase plays an important rôle in the auto-digestion, or self-fermentation of the yeast.

Coagulating Enzymes.—The presence of a coagulating enzyme in yeast juice was proved by Rapp and in extracts obtained by treating yeast with chloroform under pressure at 60°. It coagulates boiled milk, acts towards alkalies, acids and salts as rennet, and is destroyed in solution by heating for two hours at 65°; very resistant when dry, remains efficient in juice for months; does not dialyse.

Oxidising Ferments.—Effront first presumed the presence of such an enzyme in yeast, as heat is generated when air is passed

through finely-ground yeasts and yeast juice. Gruess established its oxidizing action upon tetramethyl-1-4-diamidobenzene (violet); alcohol weakens it; heat (60 to 65°.) destroys its action.

According to Loew's investigations, there also exists in yeast the enzyme called *catalase*, which he claims to be capable of decomposing hydrogen peroxide with the formation of oxygen.

The presence of *reducing enzymes* is indicated in yeast juice by the generation of nitrogen from nitrites, of hydrogen sulphide from sulphur and thiosulphates, as well as the reduction of iodine to hydriodic acid. The optimum temperature is 40°. The reduction of methylene blue is especially of an enzymic character.

. **Fermenting Enzyme.**—E. Buchner first showed (1897) the existence of an enzyme capable of splitting sugar into alcohol and carbonic-acid gas, and gave it the name "Zymase."

Zymase is contained, in addition to the other enzymes mentioned, in the juice obtained by means of hydraulic pressure from the yeast previously ground in mixture with quartz and infusorial earth; the zymase can also be extracted by water or glycerol from yeast that is previously killed with ether or acetone and then finely ground. Bottom yeasts are generally more suitable for the production of zymase.

The yeast juice ferments: dextrose, fructose, maltose, sucrose quickly, raffinose slowly, glycogen and starch very slowly, galactose very little. Lactose, arabinose and mannose are not fermented.

The zymase does not dialyse and its active power is destroyed in the juice at 40 to 50°; it acts much more slowly than other enzymes; it is precipitated by alcohol and ether together with other substances. It may be evaporated to dryness at low temperature without materially injuring its efficiency; the residue may be heated to 85° for 8 hours without harm; it may be preserved for 1 year without losing its fermentative energy. Its deterioration in the juice is due to the presence of endotryptase.

The action of zymase is increased by weak alkalies, such as potassium carbonate or sodium hydrogen phosphate. A temperature of 28 to 30° causes the quickest action, but the highest fermenting power is attained at 12 to 14°. In 30 to 40% sugar solutions zymase produces the largest percentage of carbon dioxide, but the speed of fermentation is highest in 10 to 15% solutions. Thirty to 40% sugar solutions generate 0.8 grm. of carbon dioxide within 96 hours. The yeast juice retains its fermentative power even in a dilution of 1.25.

Antiseptics do not materially influence the action of zymase; it may be preserved without injury by toluene, chloroform, sugar and glycerol. The proportions of carbon dioxide and alcohol produced by fermentation are approximately equal; succinic acid and glycerol are apparently not formed. Up to the present, zymase has never been obtained in the pure state; a preparation of active fermentative power may be made, according to Buchner, Albert, and Rapp, from "preserved yeast," ("Dauerhefe") made by bringing yeast into ether or acetone or by heating yeast in a stream of hydrogen. Such yeast finely ground with sand can be used for the production of powerful fermentative agents. The percentage of zymase in yeast varies; it increases if the yeast is stored at low temperatures.

Variation of Saccharomycetes.—The practical application of Hansen's pure culture system is based upon the assumption that pure culture yeast does not suffer any physical changes in practice. At times changes have been observed in such yeasts, which were only temporary, but Hansen succeeded in cultivating varieties and types with permanent characteristic properties.

The Circulation of Yeast in Nature.—The normal source and origin of yeast are the damaged surfaces of sweet juicy friuts, the juice of which forms the natural and best nutrient for their propagation. Rain washes the yeasts to the ground, where they remain during winter and spring, whence they are again transferred to their summer breeding places. Insects are active factors of transferring and distributing the yeast cells. Soils of orchards are especially rich in yeasts.

Important Yeast Types of the Brewing, Distilling and Wine Industries.—Culture yeasts are such that have been cultivated for long years in the fermentation industries, possessing certain qualities which make them especially adapted and available.

CULTURE YEASTS.

The following are those most frequently mentioned in literature:

1. *Saccharomyces Cerevisiæ.*—Hansen, from English and Scotch breweries; a vigorous beer top yeast.
2. *Carlsberg Bottom Yeast,* 1.—Hansen.
3. *Carlsberg Bottom Yeast,* 2.—Hansen.
    No. 1 produces very stable beer, not so readily clarifying.
    No. 2 beers not so stable, but with better clarification.

4. *Four Culture Yeasts* from Munich Station (described by Will). Tribes 93, 2, 6 and 7; the first two having a high fermenting power, tribe 6 with a medium, and tribe 7 with a low fermenting power.

5. *Distillery Yeast, II*, Berlin, isolated from a distillery in West Prussia, is a top yeast of the Frohberg type, suited for fermenting highly concentrated mashes, difficult to ferment and possessing great power of resistance to high alcoholic content.

6. *Berlin Race, V*, mostly used for the manufacture of compressed yeast.

7. *Wild Yeast.—Saccharomyces Pastorianus, I, II, III.*—Hansen. Sausage-shaped cells, disease ferments in beer. I imparts a bitter taste and odor; III causes turbidity. I may give a good product in the preparation of wine. All occur in air. II and III are top yeasts, I is a bottom yeast.

8. *Saccharomyces ellipsoideus, I and II.*—Hansen. Yeast I.—A wine yeast, found by Hansen on the surface of ripe grapes in the Vosges district, cells have an ellipsoidal shape; found to be useful and active in wine fermentation. Yeast II is a dangerous disease yeast for breweries causing turbidity. Two similar types have been isolated by Will.

9. *Saccharomyces ilicis* (bottom yeast) and *S. aquifolii Grönlund* (top yeast) found on fruit of Ilex aquifolium. They produce bitter and disagreeable taste in worts; cells mostly spherical in shape.

10. *Saccharomyces pyriformis*—Marshall Ward. Produces alcoholic fermentation of English ginger beer, forms together with Bacterium veriforme the so-called gingerbeer plant, used for the production of an acid frothing beverage—ginger beer.

11. *Saccharomyces membranæfaciens.*—Hansen. Found in wines, also in polluted waters; generates from sugar no alcohol, but acids; propagates in the presence of 12 % alcohol; consumes maltic, acetic and succinic acids; destroys the bouquet of wine.

12. *Saccharomyces mali*, Du Clauxi, Kayser, isolated from cider; ferments invert sugar, and produces esters (bouquet).

13. *Schizoaccharomyces pombe*, found by Saare in pombe (negro millet beer) from Africa A top yeast, fermenting also dextrin; used in South American distilleries with success.

14. *Schizosaccharomyces   mellacei.* — Jörgensen.   Isolated   from
    Jamaica rum.   Greg claims to have found 8 similar species in
    Jamaica rum mashes.

15. *Schizosaccharomyces octosporus.*—Beyerinck.   Found on currants
    and raisins (Greece); ferments maltose and dextrose, but not
    saccharose, shows characteristic ascus formation.

PURE CULTURE OF YEAST AND ITS APPLICATION
IN PRACTICE.

Pasteur showed, that bacteria could cause disease and detrimental
effects in these industries, and that one of the principal sources of in-
fection in the brewery was the open coolship.   His proposal to replace
the coolship by closed apparatus was not universally approved and
did not find any practical recognition.   The reason is that this method
does not prevent beer diseases, as another and very dangerous
source of infection still exists, namely, the employment of impure
yeast.

Pasteur had advocated a cleaning of yeast by means of tartaric acid,
which kills part of the bacteria, but he at that time did not know the
existence of the abnormal or disease yeasts and, as Hansen proved
later, the addition of tartaric acid favored the development of these
disease yeasts.

Hansen proved in 1879 that numerous abnormal phenomena were
directly caused by yeasts which are contained in the pitching yeast
besides culture yeasts.   He showed that the culture yeast does not
consist of a *uniform* species, but of many varieties, of which each one
imparts to beer peculiar properties and which employed collectively
may at times even cause disease phenomena.   Actuated by these in-
vestigations, Hansen founded his system of the use of pure culture
yeasts in breweries, based upon the fact that by systematic selection
a single suitable type may be made from the culture yeast which is
alone allowed to develop in the wort.

The pure cultivation of yeast is made by the following method:
A suitable quality of yeast is mixed with sterile water, and a small
quantity of this mixture is distributed into wort gelatin so that the
various cells are separated from each other on cover-glasses.   These
cover-glasses are then transferred to small moist chambers and the
development of one cell is continually observed under the microscope.

After these colonies are large enough a part of them is carefully transferred into sterile wort for further propagation. In this manner an absolutely pure culture is obtained from a single cell; these cells are examined as to their action in wort and the most suitable or appropriate species are selected for use in practice. Hansen showed that the properties of the yeasts in practice are not subject to variation in a serious degree, and that some varieties quickly assume their old characteristics.

The so-procured culture yeast is then transferred to larger quantities of a nutrient wort, and in a short time sufficient yeast is propagated for a large quantity of wort. The time and period of stability and purity of such a cultivated yeast differs according to general conditions, seasons, etc., and the resisting powers of the different pure culture species differ considerably.

It is, therefore, required, to introduce new pure-culture yeast periodically into a brewery; as soon as the biological tests and control show any deterioration of the pitching yeast, it should be renewed. The production of large quantities of pure cultivated yeast is accomplished in the so-called pure-culture apparatus in breweries and institutes devoted to fermentation industry in the manner described by Hansen.

The apparatus provides for aeration of the sterilised wort with filtered air; the wort is continually fermented and the yeast sediment is retained. Hansen introduced personally the production and application of pure-culture yeast in bottom fermenting breweries, and the largest plants in the world work according to his system, which has abolished all empiricism and replaced it by absolutely safe working methods in practice. The pure culture of top fermenting yeast was first applied in practice by Jörgensen in 1885.

Spirit and Compressed Yeast.—The pure-culture system has also found recognition by these industries owing to the efforts of Prof. Lindner in Berlin. He has introduced Race II for almost all distilleries in Germany with good results. In the United States they are only used to a limited extent.

Recently, pure cultures of lactic acid have been used together with pure-culture yeast in the distilling industry in order to prevent the harmful butyric acid fermentation. The pure culture has also been used for the manufacture of compressed yeast, particularly by the efforts of Lindner who recommended Race V for this industry.

PURE CULTURE IN WINE INDUSTRY.

This has been introduced especially since Wortmann has proved by his searching investigations that different wine-yeast types are capable of yielding most different products in regard to acidity, bouquet, as well as taste. It was also expected that yeasts from different localities would impart to any must some characteristics, but it is found that the taste and bouquet are dependent to a much larger degree upon the type of grapes, the soil, degree of ripeness, and some other factors. The bouquet substance and flavor of yeast are of a volatile nature; the use of foreign wine-yeast types has, therefore, been abandoned, and pure cultures are mostly made from the yeast types found on the native grapes and in the produced wines.

The main advantage of pure culture in wine manufacture is the rapid and vigorous fermentation before the foreign germs and wild yeasts, especially *apiculatus* or the equally dangerous acetic-acid bacteria, are capable of any vigorous development. Pure-culture wines also clarify quicker and better, and the bouquet of the young wines is generally purer.

Owing to the extremely short season of wine fermentation and the great variability of yeast types for the wine manufacture, the pure cultures are not cultivated in large apparatus as in the brewing industry, but the yeasts are fermented in small quantities by establishments specially devoted to this work. The wine producer propagates this yeast in about 2.5 to 3 gallons (10 to 12 liters) of pure boiled must and adds this to the bulk of his must as soon as it is in a vigorous stage of fermentation.

Very good results have been obtained in the manufacture of sparkling wines by Wortmann, who isolated races having the characteristic property of forming a solid sediment on the cork and producing but very little turbidity.

Seifert has introduced the pure culture especially for production of sweet wines, which are distinguished by their resistance towards high concentration of sugar and alcohol.

CIDER MANUFACTURE.

In the production of this beverage pure cultures have already been introduced successfully by Wortmann, and Kramer. Jörgensen, Kayser and Nathan have investigated their use in this field and rec-

ommend the same procedure as in the wine manufacture. The results have been satisfactory, the yeasts imparting to the cider a more or less vinous taste and flavour.

YEAST IN BAKING.

Saccharomyces cerevisiæ generates in the dough carbon dioxide and alcohol from the dextrose and maltose formed in bread during the raising and baking process. The alcohol assists the carbon dioxide in its raising power of the dough, causing the sponginess of bread, owing to the fact that it is mostly volatilised in baking. Top fermenting yeast was mostly used formerly; the bottom fermenting yeast is very slow in action and bitter; both kinds of yeast have now been entirely replaced by the so-called "compressed yeast."

The yeast mash forms an abundant yeast foam which is skimmed off, washed, watered and deprived of most of its water by filter presses or centrifuges.

Air yeast is produced from yeast mash in large fermenting vats by aerating it with sterilised air. After a fermentation of 20 hours, the yeast is washed and pressed in filter presses.

Good compressed yeast is of a light, pale yellow color, somewhat crumbly, not slimy, and of a pleasant odour. It must be protected from light and air, and kept at low temperatures; it should be even in texture, should have no sourness, an apple or fruity, not cheesy odour, and should not exhibit any dark specks and streaks. It is sometimes mixed with starch, but this is not necessary, as the machinery of to-day removes the water sufficiently. The former occasional addition of gypsum and chalk as adulteration is hardly met with to-day.

At times bottom yeast from which the bitter taste has been removed is added to compressed yeast; this may be recognised by the absence of spore colonies and the remnants of organic hop particles, especially the lupulin or oil glands of hops.

Authorities seem to differ in regard to the addition of starch, but most of them state that starch is added for the purpose of reducing cost, and consider it a reduction of quality and a distinct adulteration. The addition of starch should be stated on the label.

Compressed yeast should be used when fresh; it easily becomes stale and deteriorates; it is generally wrapped in tinfoil and kept cold; texture and fracture should be uniform and even.

Dry yeast is classed by Leach as a product obtained by mixing fresh yeast with starch or meal into a stiff dough which is subsequently dried at low temperature and under reduced pressure; this preparation is said to keep for a long time, and although the cells are largely rendered inactive by the drying process, they do not lose their power of fermentation.

PHYSICAL EXAMINATION OF YEAST.

Examination of the physical charactertistics of yeast is often made by the chemist, and is no doubt of some practical value, although the results cannot be claimed as decisive. Among the more important physical tests the following may be mentioned:

1. The yeast should form a solid sediment after fermentation.
2. The yeast should be crumbly, not doughy or slimy.
3. Odour should be pleasant, pure, clean, aromatic, not sharp, repulsive, or cheesy, taste slightly and agreeably bitter.
4. Colour should be light yellow (sometimes darker from beers with deeper colour.
5. Mixed with cold water it should settle rapidly and form a solid compact sediment.

For a more extensive and comprehensive examination we must resort to a microscopical and biological examination.

MICROSCOPICAL EXAMINATION.

Some of the sample is mixed with sterilised water to a milky fluid, some of this is brought by means of a sterile glass rod upon a slide, the cover-glass put on and the yeast examined with a power of 600 to 800. Thus the form, shape and size of the cells are observed, the condition of the cell membrane and the cell contents (protoplasm); the presence of *dead yeast* cells is determined by adding to another sample of the water and yeast mixture a drop of stain, such as methyl violet, eosin, or fuchsin. A solution of the stain is made by dissolving 1 grm. of the dye in 160 c.c. of water and 1 c.c. of alcohol. Living and active cells do not absorb the stain so readily, while dead cells are immediately stained.

Lindner recommends the following stain and method: 1 part of powdered indigo is rubbed with 4 parts of concentrated sulphuric

acid, allowed to stand 24 hours, diluted with 20 to 30 times its volume of water, heated to 50° and neutralised with calcium or sodium carbonate. To a sample of yeast 1 drop of staining solution is added. allowed to act a few seconds, diluted with a weak sucrose solution, the whole thoroughly mixed and a drop examined on the slide under a cover-glass. Old and dead yeast cells exhibit a thickened membrane. A good yeast should contain no more than 3 to 4 % of stained cells.

The microscope field shows also the presence of foreign ferments, the addition of other substances, such as starch, as well as contamination with mould spores and bacteria. The detection of the latter is simplified by adding a 5 % sodium hydroxide solution to the preparation.

It is obvious that a yeast should be as free as possible from any contaminating, foreign organisms, such as lactic, acetic, and butyric acid bacteria. It should also be free from any so-called wild or abnormal yeasts (as described below). These are generally recognized by their variation and distinct difference in shape, although the presence of abnormal yeasts (not culture yeasts) should always be corroborated by the so-called plate cultures and spore methods as described in special works by Hansen and Klöcker.

The formation of ascospores as studied by Hansen proceeds under the following prevailing conditions:

1. Abundant air must be admitted to the yeast cells; propagation occurs on a moist surface (gypsum block).
2. Only young, vigorous cells produce these spores.
3. The optimum temperature is 25° for the most known species.
4. Some few species form spores, even if present in fermenting, nutrient solutions.

The fundamental differences between the varous species are especially the temperature and the time necessary for spore formation, i. e., S. cerevisiæ is distinguished from the wild yeasts in that the latter form ascospores in a much shorter time under the same conditions and temperature. The ascospores of the culture yeast are also much larger than those of wild yeasts.

CHEMICAL TESTING OF YEAST.

The water and ash of a yeast are determined according to the standard methods of food analysis.

The general and most important criterion for the valuation of yeast in the fermentation industry is the so-called fermentative and raising power which is especially required for the valuation of compressed yeast.

By activity or energy of fermentation is understood the degree of intensity with which a yeast is able to decompose a certain quantity of sugar within a specified time. It not only serves to distinguish the various yeast species from each other, but is also useful in establishing a criterion for the different physiological conditions of any given species of yeast.

The methods used for this determination are based upon the estimation of the carbon dioxide generated from a sugar solution. The amount is ascertained either by weight or by volume. The former method, according to Meissl, is especially applicable if only small amounts of yeast are at disposal. The latter (Hayduck and Kusserow) is principally used to examine yeasts in practice.

Meissl's Method.—This method determines the weight of carbon dioxide which is generated by 1 grm. of yeast within 6 hours at 30° from a solution in ordinary tap water of a mixture of 400 grm. of pure sucrose, 25 grm. of ammonium phosphate and 25 grm. of potassium phosphate.

According to Meissl, a "normal" or "standard" yeast is one that liberates under these conditions 1.75 grm. of carbon dioxide, and the energy of the yeast is then figured as 100.

The fermenting power is then found according to the following equation:

$$1.75 : n = 100 : x \text{ in which}$$
$$n = \text{quantity of carbon dioxide.}$$

Prior found according to this method the following values for the various species:

Carlsberg bottom yeast, No. 1,	136.40
Carlsberg bottom yeast, No. 2,	106.13
S. pastorianus I,	155.48
II,	280.72
III,	202.20
S. ellipsoideus, I,	285.76
II,	219.03

FERMENTING POWER.

Meissl's Method.—Of the above mixture 4.5 grm. are dissolved in 50 c.c. of tap water; it is also suggested to use gypsum water by mixing 15 parts of a saturated solution of calcium sulphate with 35 parts of distilled, aerated water.

The solution is introduced into an Erlenmeyer flask of about 100 c.c. capacity, together with exactly 1 grm. of yeast, which should be thoroughly distributed so as to form a uniform mixture without lumps. The flask is then fitted with a doubly perforated rubber stopper, having 2 tubes, one of which is bent and passes nearly to the bottom of the flask and fitted at the other end with a rubber tube and glass plug, while the other is connected with a calcium chloride tube. The whole apparatus thus arranged is weighed accurately and kept in a thermostat or water-bath at 30° for 6 hours. At the end of this time it is removed from the thermostat, quickly cooled in cold water, the rubber tube and glass plug taken off and the remaining carbon dioxide drawn out by suction; the glass plug and rubber tube are replaced and the flask carefully weighed as before. The loss of weight is equal to the quantity of carbon dioxide generated by the fermentation of the sugar owing to the activity of the yeast. A good compressed yeast should have at least 75 to 80 % of fermentative energy or fermenting power.

Methods of Hayduck and Kusserow.—In both methods the carbon dioxide generated by a given quantity of yeast in a certain sugar solution is measured by volume. Hayduck measures the volume of carbon dioxide directly, while Kusserow ascertains the quantity of water displaced by it and measured in a graduated cylinder, which serves as a receptacle.

Hayduck uses an apparatus similar to Scheibler's carbon dioxide apparatus consisting essentially of a 500 c.c. burette divided into c.c. and connected by a rubber tube with a large glass bulb.

For both methods the following procedure is prescribed: 40 grm. of pure sucrose are dissolved in 400 c.c. of water and this solution brought to 30°. 10 grm. of compressed yeast are intimately mixed with successive portions of the solution in a porcelain dish until no more lumps are visible. The mixture is introduced into a 1000 c.c. flask and the porcelain dish is thoroughly rinsed with the remainder of the sugar solution until all of the solution is put into the flask; the whole is now thoroughly shaken

and placed into a water-bath at 30° allowed to stand open in the bath for 1 hour, while carbon dioxide freely escapes.

The flask is then connected by means of glass and rubber tubing with the apparatus filled with water to the zero mark. In order to avoid any absorption of carbon dioxide by the water, a little petroleum is used floating in a very thin layer upon the water.

After 1/2-hour connection the vent is closed, the flow of carbon dioxide is shut off and the measured volume of gas is read off in the burette after the water in the burette and the glass bulb are brought to an equal level. The c.c. of carbon dioxide may express directly the fermenting power of the yeast; or the weight of sucrose, which has been decomposed by 100 grm. of yeast, may be calculated by multiplying the found figure by 0.03841; as 342 grm. of sucrose will give 176 grm. carbon dioxide, 1 c.c. of which weighs 0.001977 grm. The weight of sucrose necessary for the production of 1 c.c. carbon dioxide =

$$\tfrac{342}{176} \times 0.001977 = 0.003841.$$

Kusserow's Method.—In this method exactly the same quantities of materials are used, but after the flask has been standing in the water-bath at 30° for an hour it is connected with another flask of exactly the same capacity absolutely full of water. This flask has a doubly perforated stopper through which pass two glass tubes, both bent at right angles, going to the bottom of the flask, and bent again at right angles under which is standing a graduated cylinder of 500 c.c.

After an hour the gas is allowed to pass into the second flask; it forces the water out and this is collected in the graduated cylinder.

A good baker's yeast should have according to this method a fermenting power of 250 c.c.

Kusserow also estimated the fermenting power after the first and second half-hour; during the first period a good yeast should give 50 c.c. and in the second half-hour 150 c.c. of carbon dioxide.

Acidity.—5 to 6 grm. of yeast are mixed with distilled water, introduced into a flask and titrated with normal sodium hydroxide using phenolphthalein as an indicator. The acid degree is expressed in mg. of the alkali for 100 grm. of yeast or in % of lactic acid, 1 c.c. normal alkali = 0.09 grm. lactic acid.

Starch.—The addition of starch to yeast before pressing has long been customary, basing its use upon the drying qualities of starch.

The best grades of compressed yeast contain about 5 %., some as high as 50 %. The larger amounts are looked upon as an adulteration. **Estimation of Starch.**—See under starch.

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NEUTRAL ALCOHOLIC DERIVATIVES.

By HENRY LEFFMANN.

The neutral derivatives of the alcohols include a number of important bodies, of which chloroform, chloral, ether, some esters, formaldehyde and acetaldehyde are prominent examples.

ETHER.

Ethyl Ether. Ethyl Oxide.

When used as a name "ether" always signifies ethyl ether. When employed generically the word has a wider signification.

Ether is obtained in many reactions, but usually by distilling alcohol with strong sulphuric acid, hence the common name "sulphuric ether"—a name that belongs to ethyl sulphate. The reaction consists in the production of ethyl hydrogen sulphate (sulphovinic acid), and this at a higher temperature acts on a second molecule of alcohol with formation of ether.

Theoretically, a limited quantity of sulphuric acid is capable of converting a large quantity of alcohol. Advantage is taken of this in practice, but the formation of secondary products ultimately interferes. The first distillate contains several impurities, among which are alcohol, water, sulphurous and acetic acids and oil of wine. By addition of water much of the alcohol may be eliminated, the ether forming a layer on the surface. The acids and water may be removed of by agitation with potassium carbonate, and the ether obtained nearly pure by redistillation.

Ether is a highly volatile, colourless, odourous limpid liquid, of pungent, sweetish taste. It boils at 35° and has a sp. gr., according to Mendelejeff, of 0.7195 at 15°, or 0.7364 at 0°. It solidifies at —129° to a white crystalline mass, which liquifies at —117.4°. It is sparingly soluble in water, less so in glycerol; the solutions are neutral to ordinary indicators. With alcohol, chloroform, benzene, petroleum spirit, fixed and volatile oils, it is miscible in all proportions.

Ether dissolves resins, fats; many alkaloids; phosphorus, bromine, and iodine; ferric, mercuric and auric chlorides; and mercuric (but not mercurous) iodide. In the air, it oxidises very slowly to acetic acid. Both the liquid and vapor burn freely with a white flame.

Commercial Ether.—Commercial ether contains water (1 part of water dissolves in 35 of ether) and considerable quantities of alcohol. "Ether," British Pharmacopœia, is described as having a sp. gr. of 0.735, and containing not less than 92% by volume of real ether.

"Ether," United States Pharmacopœia, is a liquid composed of about 96% by weight ethyl oxide and about 4% of alcohol containing a little water. Sp. gr. —0.716 to 0.717 at 25°.

"When 20 c.c. are shaken with 20 c.c. of water previously saturated with ether, the upper layer upon separation should measure not less than 19.2 c.c. If 10 c.c. of ether are shaken occasionally during one hour with 1 c.c. of potassium hydroxide solution, no colour should be developed in either liquid (absence of aldehyde)."

Water or alcohol in ether tends to raise the b. p. and increase the sp. gr. of the liquid.

Dr. Squibb found the following sp. gr. for mixtures of ether (0.71890) with alcohol (0.82016) (=90.94% by weight of absolute alcohol); the observations being taken at 15.5°, and compared with water at the same temperature taken as unity:

Percentage of ether by weight.	Specific gravity.	Percentage of ether by weight.	Specific gravity.	Percentage of ether by weight.	Specific gravity.
99	0.72021	89	0.73298	79	0.74495
98	0.72152	88	0.73428	78	0.74612
97	0.72284	87	0.73547	77	0.74729
96	0.72416	86	0.73666	76	0.74846
95	0.72541	85	0.73785	75	0.74975
94	0.72666	84	0.73904	74	0.75104
93	0.72792	83	0.74022	73	0.75233
92	0.72918	82	0.74141	72	0.75362
91	0.73043	81	0.74260	71	0.75492
90	0.73168	80	0.74378	70	0.75623

Absolute ether forms a clear mixture with any proportion of oil of copaiba. If alcohol or water is present an emulsion is formed when shaken with a considerable proportion of the oil. Anhydrous ether also forms a perfectly clear mixture with an equal bulk of carbon

disulphide, but if the smallest quantity of water is present the mixture is milky.

Tannin is not affected by anhydrous ether, but it deliquesces to a syrup if a small proportion of alcohol or water is present.

The most delicate test for alcohol in ether is that of Lieben, depending on the formation of iodoform. The method is described on page 105. Careful purification is necessary to obtain ether which will not respond to this test; mere keeping in presence of moisture generates traces of alcohol sufficient to produce the reaction.

Several chemists have pointed out that rosaniline acetate is insoluble in pure ether or chloroform, but imparts more or less colour to these when alcohol or water is present.

When a sample is well agitated with dry calcium chloride to remove alcohol and water, it loses the power of dissolving the rosaniline salt, becoming tinged very faintly when shaken with it.

To utilize this fact for the estimation of small quantities of alcohol in ether, Allen suggested the following process. A minute quantity of powdered rosaniline acetate is placed in a narrow test-tube, 10 c.c. of the ether added, the tube corked, and the whole agitated. If the ether is anhydrous, the colouration of the liquid will be almost inappreciable. If the colouration is considerable, 10 c.c. of ether treated with calcium chloride is placed in another tube of the same bore as the first, adding the dye as before. o.1 c.c. of alcohol is then added from a finely divided burette, and the mass shaken. If this quantity of alcohol is insufficient to produce a colour equal to that of the sample, further additions of alcohol must be made until the liquids have the same depth of colour. The tint is best observed by holding the two tubes side by side in front of a window and looking through them transversely. The use of a piece of wet filter-paper behind them facilitates the observation. It is well to permit the alcohol to drop right into the ether, and not allow it to run down the sides of the tube, as in the latter case it will dissolve any adherent particles of dye, forming a solution which will be precipitated on mixing with the ether. It is also not advisable to dilute the sample with pure ether, so as to reduce the color to that of a standard tint. In practice, each o.1 c.c. of alcohol added from the burette may be considered as indicating 1% of impurity in the sample; the error thus introduced is insignificant when the percentage of alcohol is small. The method is unsatisfactory when the alcohol exceeds 5% of the sample, owing to the intensity of the colour.

The results are within 0.25 % of the truth. Occasionally the tints of the two liquids are not readily comparable, but on placing the tubes for a few minutes in cold water, this difficulty is overcome. It has been pointed out by E. R. Squibb, that this test fails to detect less than 0.2 % alcohol, but allows the recognition of very minute traces of water.

Ether free from alcohol is soluble in eleven times its measure of water. Agitation with water extracts any alcohol it may contain, and thus diminishes the volume of the ether. With certain precautions, this method may be used in connection with the above test. The following are the details of the procedure that Allen devised: A small quantity of rosaniline acétate is placed in a separator which is then filled with water and a small proportion of ether, and the whole agitated. A coloured etherised water is obtained, in which ether is quite insoluble, while alcohol readily dissolves. 10 c.c. of the etherised water are run into a glass tube holding about 25 c.c., and having divisions of 0.1 c.c.; 10 c.c. of the sample of ether are next added, the tube corked, and the whole well shaken. On the ether rising to the surface, its volume can be easily read off. Any reduction in its volume is due to admixture of alcohol. Each 0.1 c.c. lost represents 1 % of alcohol. If the alcohol does not exceed 20 % the ether will be colourless, and the result of the experiment will be correct; but if the alcohol is much above 20 % the ether will be coloured, and the result below the truth. The absence of colour, therefore, in the ethereal layer, indicates the accuracy of the experiment. If the ether is coloured, an accurate result can still be obtained by adding 5 c.c. of anhydrous ether, and again agitating. It is better, however, to dilute a fresh portion of the sample with an equal bulk of pure ether, and use the diluted sample instead of the original. By proceeding in this manner the proportion of alcohol in mixtures of that liquid with ether can be ascertained within 1 or 2 % with great facility. The process has been verified up to 60 % of alcohol.

In all cases the proportion of alcohol must be deduced from the reduction in the volume of the ether, and not from the increase in that of the aqueous liquid. Care must be taken to prevent any volatilisation of the ether.

Some of the objectionable impurities in ether may be detected by allowing a small amount not less than 10 c.c. to evaporate at ordinary temperature on filter-paper lying in a flat dish. The odour of the last portion should be examined.

Good ether gives no unpleasant odour. Allen noted samples of ether which liberated iodine from potassium iodide, an action which he ascribed to the presence of ethyl nitrite.

Methyl Ether.—When methyl alcohol is heated with sulphuric acid it yields methylic ether, which is a gas condensible only at a very low temperature, and the solution of which in ordinary ether possesses anæsthetic properties. Ether prepared from methylated spirit is known as "methylated ether," but this should not be used in medical work.

Spirit of Ether, British Pharmacopœia, is a solution of about 28 parts of ether in 72 of rectified alcohol. The United States Pharmacopœia preparation is prepared by mixing 325 c.c. of official ether and 675 c.c. of official alcohol. *Compound spirit of ether*, United States Pharmacopœia, is made by substituting 25 c.c. of "ethereal oil" for an equal quantity of the alcohol in the simple spirit, otherwise as in that substance.

ESTERS—COMPOUND ETHERS.

This term is applied to the products of acids on alcohols with elimination of water. They are analogous, therefore, to the ordinary inorganic salts:

Esters can be produced in many ways. The following are general methods:

By the action of concentrated acids upon anhydrous or concentrated alcohols.

By distilling an alcohol with strong sulphuric acid and a salt of the acid the radicle of which is to be introduced.

By dissolving an acid in an alcohol and passing hydrochloric-acid gas into the solution.

By reaction between the iodide of the radicle and the silver salt of the acid.

The esters are analogous to salts, but they rarely react directly with the ordinary tests for the contained radicles.

As a class, they are mostly colourless, volatile liquids slightly soluble in water, but miscible in all proportions with alcohol and ether.

The fats, fixed oils and waxes consist largely of esters, but are considered in a separate section on account of differing much in physical characters and practical applications from the esters here described.

A method of assay applicable to most esters is "saponification," by

which is meant decomposition by means of sodium hydroxide or potassium hydroxide. By this action an alcohol and a salt of the acid radicle present is formed. High-pressure steam decomposes many esters by simple hydrolysis, often erroneously termed saponification. The two actions are shown in contrast in the following equations:

The saponifying substance is usually dissolved in alcohol or glycerol. The latter liquid, suggested by Leffmann and Beam, is especially applicable to the saponification of the complex esters contained in fats, fixed oils and waxes and its preparation and use is described in connection with the analysis of that class of substances. Aqueous solutions are very slow in action and the direct action of high-pressure steam is suitable only to operations on the large scale.

Many esters are hydrolyzed at ordinary temperatures by some enzymes. Such an enzyme is found in the secretion of the pancreas; another very active one in the castor bean. Practical use of the latter enzyme has been made in the decomposition of oils on the large scale.

The following are the details of the ordinary process of saponification which is practically identical with that of Koettstorfer for the examination of fats:

A volume of 50 c.c. (measured with the greatest attainable accuracy) of a solution of potassium hydroxide (about 60 grm. in 1000 c.c. of alcohol) is introduced into a strong bottle holding about 100 c.c. A weighed quantity of the ester (from 4 to 6 grm.) is then added in such a manner as to avoid loss. This may be contained in a small glass bulb, or a known weight dissolved in pure alcohol may be added. The bottle is closed with an india-rubber stopper firmly secured by wire, heated to 100° for half an hour, allowed to cool, opened, a few drops of phenolphthalein solution added, and the liquid at once titrated with normal acid. A blank experiment is made by heating 50 c.c. of the reagent alone for half an hour and titrating. The *difference* between the acid required in the blank experiment, and that in which the ester was present, is acid corresponding to the alkali neutralised by the ether. Each cubic centimetre of normal acid represents 0.0561 grm. of potassium hydroxide, or, in other words, each 1 c.c. of *difference* between the measure of the acid originally employed, and that used in the blank experiment represents *one equivalent in* mg. of the ester present.

As an example: Suppose that 45 c.c. of normal acid were employed in the blank experiment, and that 8 c.c. were required after saponification. The difference of 37 c.c. represents the alkali taken for the

decomposition of the ester. As each centimetre of this contains 56.1 mg. or one equivalent in mg. of alkali, it follows that the sample contained a number of mg. equal to 37 times its equivalent. Supposing the weight of ester was 4.810 grm., then its equivalent would be 4810/37 = 130. Of course, the equivalent thus found is identical with the molecular weight, 1/2 or 1/3 of the same, according to the constitution of the ester.

Conversely, if the equivalent of the ester known to be 130, the weight of it present in the quantity of the sample taken will be 130 × 37 = 4.810 grm.

This method often furnishes valuable evidence of the purity of the substances examined. An elementary analysis would scarcely detect 10% of ethyl alcohol in ethyl acetate, or of amyl alcohol in amyl acetate, but the saponification process would indicate these with certainty.

After decomposing the ester and titrating with acid, further knowledge may be obtained as follows:

The free alcohol is removed by distilling or evaporating the liquid after rendering it slightly alkaline. The residue is treated with an amount of sulphuric acid double that sufficient neutralise the alkali originally added (*i.e.*, to produce potassium hydrogen sulphate), and the liquid is distilled. The acid of the esters will be liberated, and, if volatile without decomposition, will pass more or less perfectly into the distillate, where it may be further examined. It is obvious that if such operation is to be applied sulphuric acid should be used in the titration.

This method may be employed for the estimation of chloroform and chloral hydrate when n alcoholic solution.

Each c.c. of *difference* in the amounts of normal sulphuric acid required will represent 0.0299 grm. of chloroform or 0.0331 grm. of chloral hydrate.

For estimation of esters in spirits, see page 195.

Many fruits owe their flavour to mixtures of esters, principally those containing the alcohol radicles of the methyl series. It has become a common practice to use artificial esters for imitating such flavours. The complete analysis of the mixtures sold is often impossible at present, but special ingredients may be detected. The following table shows some of the constants of the more important esters of the series mentioned above. For important salicylic and benzoic esters, see Vol. II, part 3, of this work.

The data in this table were compiled from several sources, principally Fehling's *Handwörterbuch d. Chemie;* Meyer and Jacobsen's *Lehrb. d. org. Chem.*, and Landolt and Börnstein's *Tabellen*. In many cases the numbers must be considered as provisional, as the purification of these esters is difficult; moreover, all compounds of this series containing more than three carbon atoms in either radicle, present isomeric forms, which in the higher members are quite numerous; these forms will differ in b. p. and sp. gr. The table will serve, however, to show some of the general characters. A few esters require description at considerable length.

	B. p.; under standard pressure, unless otherwise stated.	Sp. gr.; at 0°, unless otherwise noted.
Methyl formate...............	33°	0.940
Methyl acetate...............		0.956
Methyl butyrate..............	96°–102°	0.920
Methyl chloride..............	—23.7°	0.952
Methyl bromide..............	4.5°	1.732
Methyl iodide...............	45°	2.293
Methyl sulphate.............	187°–188	1.327
Ethyl formate...............	54.3°	0.945
Ethyl acetate...............	74.6°	0.8981
Ethyl butyrate...............	115°–121°	0.904
Ethyl nitrate................	87°	1.109 at 20°
Ethyl nitrite................	17°	0.900 at 15.15°
Ethyl sulphate..............	96° (15 mm.)	1.184 at 19°
Ethyl chloride..............	12.2°	0.925 (0°/0°)
Ethyl bromide..............	38.4°	1.473 (0°/0°)
Ethyl iodide................	72.3°	1.935 at 20°/20°
Isoamyl formate.............	116°	0.874 at 21°
Isoamyl acetate.............	148°	0.8963
Isoamyl butyrate............	176°	0.852 at 15°
Isoamyl nitrate.............	147°–148°	0.999 at 20°
Isoamyl nitrite.............	94°–95°	0.902
Isoamyl chloride............	101°	0.813
Isoamyl bromide............	121°	1.236
Isoamyl iodide	148°	1.468

These constants are of less practical value than is usual with such data, as most esters are encountered in commerce in more or less complex mixture, so that determinations of b. p. and sp. gr. have little indicative value. The following examples of formulas for commercial artificial flavours are taken from a recent publication. The propor-

tions are parts by volume added to 100 parts of cologne spirit (see page 112).

Pineapple Flavor.—Chloroform, 1 part; aldehyde, 1 part; ethyl butyrate, 5 parts; amyl butyrate, 10 parts.

Strawberry Flavor.—Ethyl nitrite, acetate and formate, each 1 part; ethyl butyrate, 5 parts; amyl butyrate, 2 parts; amyl acetate, 5 parts.

Raspberry Flavor.—Ethyl nitrite, acetate, formate, butyrate, benzoate, œnanthylate and sebacate; methyl salicylate; amyl acetate and butyrate, aldehyde: each 1 part.

In the examination of these mixtures, chloroform and allied bodies can be detected and estimated by conversion into hydrochloric acid, as described on page 275; aldehyde by the methods on pages 197 and 265; nitrite can be detected even in minute amounts by Greiss' test (page 241) and if present in notable quantity can be estimated by the standard method of assay (page 245).

The fruit esters are usually saponified by the method given on page 232 As many of them are highly volatile, the operation must be conducted in a flask connected closely to a well-cooled reverse condenser. A freshly-prepared solution of pure sodium hydroxide in alcohol as free as possible from esters and aldehyde may be used. Small amounts of methyl alcohol may be expected, as methyl salicylate is often present. Alcohol is, of course, to be avoided as the solvent for the saponifying agent, when it is desired to detect or estimate ethyl as one of the radicles present. Water or glycerol may be substituted in such cases. The saponification with water is usually slow; a strong solution should be employed and the mixture heated for some time either in a strong, tightly-closed flask or under a reverse condenser. In all cases the condenser should be well cooled. The glycerol solution is made by mixing 20 c.c. of a 50% solution of sodium hydroxide with 180 c.c. of good glycerol.

The sodium salts resulting from the saponification may be separated by distilling the mass, care being taken not to overheat it, which would develop empyreumatic substances that interfere seriously with subsequent tests. The distillate contains the alcohols produced by the saponification, and the volatile, unsaponifiable ingredients, such as chloroform and aldehyde. By adding sulphuric or phosphoric acid in slight excess to the solid residue and again distilling, especially in a current of steam, some of the acids may be obtained in the distillate

and may be recognised by odour and special tests. It is often impossible to distil all the acids present in this way.

A general method of examining esters was devised by Chapman and Smith (*Jour. Chem. Soc.*, 1871, **19**, 477). It is based on the fact that organic bodies when oxidised in a sealed tube by a mixture of sulphuric acid and potassium dichromate yield proximate products of oxidation closely related to the radicles contained in them.

The process consists in heating a known weight of the substance in a sealed tube for some hours with an aqueous solution, containing from 3 to 8% of potassium dichromate and 5 parts by weight of concentrated sulphuric acid to every 4 of the dichromate. The following reactions were verified by the authors of the method as occurring almost in theoretic proportion.

Ethyl alcohol,	$C_2H_6H + O_2$	$= HC_2H_3O_2 + H_2O.$
Amyl alcohol,	$C_5H_{12}O + O_2$	$= HC_5H_9O_2 + H_2O.$
Ethyl acetate,	$C_2H_5,C_2H_3O_2 + O_2$	$= 2HC_2H_3O_2.$
Amyl acetate,	$C_5H_{11},C_2H_3O_2 + O_2$	$= HC_2H_3O_2 + HC_5H_9O_2.$
Amyl valerate,	$C_5H_{11},C_5H_9O_2 + O_2$	$= 2HC_5H_9O_2.$
Amyl nitrite,	$C_5H_{11},NO_2 + O_3$	$= HC_5H_9O_2 + HNO_3.$
Ethyl nitrate,	$C_2H_5,NO_3 + O_2$	$= HC_2H_3O_2 + HNO_3.$
Ethyl iodide,	$2C_2H_5I + O_5$	$= 2HC_2H_3O_2 + H_2O + I_2.$
Isopropyl iodide,	$2C_3H_7I + O_{11}$	$= 2HC_2H_3O_2 + 2CO_2 + 3H_2O + I_2.$
Ethylamine,	$C_2H_5,H_2N + O_2$	$= HC_2H_3O_2 + NH_3.$
Ethylamylamine,	$C_2H_5,C_5H_{11},HN + O_4$	$= HC_2H_3O_2 + HC_5H_9O_2 + NH_3.$
Ethyl benzoate,	$C_2H_5,C_7H_5O_2 + O_2$	$= HC_2H_3O_2 + HC_7H_5O_2.$

Compounds containing methyl yield formic acid by oxidation, but the greater part of this is further oxidised to carbon dioxide and water. Chapman and Smith (*Jour. Chem. Soc.*, 1872, **20**, 173) further showed that the process was capable of being used for investigating the structure of isomeric bodies. This is exemplified in the equation representing the oxidation of isopropyl iodide.

These methods of examining esters are of such general application that, with the aid of the table on page 234, some esters in common use may be readily identified, and even quantitatively assayed. The assay of commercial esters may usually be conducted as described under "Ethyl Acetate" A few, however, owing to their special properties or great individual importance, will be considered in separate sections.

Ethyl Acetate (Acetic Ether).—This is prepared by distilling dried

sodium acetate with alcohol and sulphuric acid. The product is purified from alcohol by agitation with a saturated solution of calcium chloride, and subsequently dehydrated by contact for some days at the ordinary temperature with recently ignited potassium carbonate, or distillation over dried sodium acetate. The use of solid calcium chloride for dehydration causes considerable loss from the formation of a compound with the ethyl acetate, decomposed on addition of water.

Ethyl acetate occurs in many wines and in wine vinegar. It is produced spontaneously in several pharmaceutical preparations, notably in a solution of ferric acetate in alcohol. It possesses considerable solvent powers, and is employed for extracting morphine and tannins from aqueous liquids.

It is a colourless, fragrant liquid having a sp. gr. of about 0.908 at 15°, and boils at 73.5 to 74.3°. It is miscible in all proportions with alcohol, ether and chloroform, but only sparingly soluble in water, requiring 8 volumes at 0°, or 9 at 15° for its solution. The solubility of water in ethyl acetate is 1 measure in 26 at 0°, and 1 in 24 at 15°. In a saturated solution of calcium chloride, ethyl acetate is but very slightly soluble, requiring 47 measures at 15°, and almost as large a proportion at 0°.

Commercial ethyl acetate is often impure. In a series of eight samples representing the products of most of the leading makers, W. Inglis Clark found proportions of real ethyl acetate ranging from 90.14 to 30.6 %; the alcohol from 7.2 to 48.0 %; the free acetic acid from a trace up to 7.0 %; while the other impurities (estimated by difference) ranged from 1.5 to 29.6 %.

For the analysis of the commercial product the following process gives satisfactory results:

Dissolve 5 c.c. in proof spirit (freed from acid by adding a few drops of phenolphthalein, and then dropping in dilute alkali until a faint pink tint remains after shaking) and titrate with decinormal alkali. Each 1 c.c. neutralised represents 0.006 grm. of free acetic acid in the 5 c.c. used.

Add to another quantity of 5 c.c. of the sample the measure of alkali that has been employed in the titration, and then saponify the neutralised liquid as described on page 232. Each 1 c.c. of normal alkali neutralised by the sample represents 0.088 grm. of *ethyl acetate* in the quantity of the sample used, or 0.046 of alcohol regenerated from the ester.

20 c.c. of the sample are mixed with 20 c.c. of water and about 12 grm.

of solid sodium hydroxide and placed in a flask with inverted condenser. After macerating for about an hour at room temperature the mass is heated at 100° for two hours, then 20 c.c. of water added and the mixture distilled until 50 c.c. are collected. The alcohol distillate is estimated in the usual way. The weight so found is divided by 4 and from the dividend is substracted the amount of alcohol produced from the ethyl acetate ascertained by the Köttstorfer method on page 232. The difference is the alcohol present as such in 5 c.c. of the sample.

By subtracting the sum of the *acetic acid, ethyl acetate* and *alcohol* found as above from the weight of 5 c.c. of the sample, the total amount of other impurities may be ascertained.

A very simple and approximately accurate method of ascertaining the proportion of real ethyl acetate present consists in agitating 10 c.c. of the sample, in a graduated tube, with an equal volume of a saturated solution of calcium chloride. The volume of the layer which rises to the surface is the quantity of ethyl acetate. The results are fairly accurate, if the water and alcohol of the sample do not together much exceed 20% by vo ume, but with larger proportions the volume that separates is sometimes notably below the real amount of ethyl acetate present. The error may be avoided in some measure by adding to the sample twice its volume ethyl acetate that has been previously treated with calcium chloride solution. 20 c.c. of the fortified sample should then be shaken with 20 c.c. of calcium chlor-. ide, when the diminution in the volume of the ethereal layer will represent the measure of impurities in $20/3 = 6.67$ c.c. of the sample.

This method is due to W. Inglis Clark. The employment of water previously saturated with washed acetic ether, and coloured with a rosaniline salt does not give satisfactory results.

The sp. gr. of ethyl acetate is not a satisfactory indication of its purity, as it dissolves alcohol, ether, and chloroform in all proportions and may be diluted with a spirit of approximately the same sp. gr. as the pure substance.

It should not contain more than a trace of free acid, and should be entirely volatile without residue, nor be blackened by strong sulphuric acid.

The United States Pharmacopœia requirements refer to "acetic ether" containing about 90% of ethyl acetate, about 10% of ethyl alcohol and a little water. Sp. gr., o 883 to 0.885 at 25°. When

evaporated spontaneously from clean unsized paper, the final odour should not suggest that of pineapple.

25 c.c. shaken in a graduated tube with an equal volume of water previously saturated with pure ethyl acetate and the liquids allowed to separate should give an upper (ether) layer measuring not less than 22.5 c.c.

A small portion of ethyl acetate poured upon strong sulphuric acid should not develop a dark ring at the point of contact of the two liquids.

Ethyl Sulphates.—The ethyl sulphates (sulphovinates) are the salts of ethyl hydrogen sulphate sometimes called ethyl sulphuric acid or sulphovinic acid.

Ethyl hydrogen sulphate (acid ethyl sulphate) is produced by the interaction of alcohol and strong sulphuric acid. The action is aided by keeping the mixture at 100° for 24 hours. The less water present, the more change occurs; but it is always far from complete. If the temperature be raised much above 100°, ordinary ether is produced, and, at higher temperatures still, ethylene (ethene) and other products appear.

From the crude acid, *barium ethyl sulphate* may be prepared by neutralising the liquid with barium carbonate, filtering off barium sulphate, and evaporating the filtrate to crystallisation. The *calcium* salt may be obtained in similar manner, and the lead salt by employing lead monoxide instead of barium carbonate.

. **Sodium Ethyl Sulphate,** may be obtained by decomposing one of the above salts with sodium carbonate, or by adding powdered sodium carbonate and alcohol, or alcoholic solution of sodium hydroxide, to the crude acid, filtering from the insoluble sodium sulphate and evaporating the filtrate to crystallisation.

Sodium ethyl sulphate (sodium sulphovinate) is a white crystalline salt of faint ethereal odour, and cooling, sweetish, somewhat aromatic taste, very deliquescent, soluble in 0.7 parts of cold water, and also soluble in alcohol, with which it is capable of forming a crystalline compound. It is insoluble in ether. At 86° it melts and becomes anhydrous; at 120° it decomposes, evolving alcohol vapour, and leaving acid sodium sulphate. It also decomposes spontaneously at ordinary temperatures, especially when in solution, with formation of sodium sulphate. The presence of a little free alkali prevents this change. The commercial salt is liable to contain barium, calcium, lead, arsenic, sulphates and other impurities. It is not unfrequently contaminated

with foreign organic matter. When pure it does not char on ignition. It has been adulterated by admixture with sodium sulphate and has been replaced by barium acetate. The last dangerous substitution would at once be detected by adding dilute sulphuric acid to the aqueous solution.

The characters of the ethyl sulphates are sufficiently indicated by the above description of the sodium salt. They are soluble in water. When heated with dilute sulphuric acid they evolve alcohol, and with strong sulphuric acid, ether. With sulphuric acid and an acetate they give a fragrant odour of acetic ether. The same result is obtained by simply heating together an acetate and sulphovinate.

Ethyl Sulphuric Acid, may be obtained in a state of purity by decomposing the barium salt by an equivalent amount of dilute sulphuric acid or a solution of lead ethyl sulphate by hydrogen sulphide. On concentrating the filtered liquid, the acid is obtained as a limpid, oily, very sour, unstable liquid of 1.31 sp. gr. It is miscible with water and alcohol in all proportions, but it is insoluble in ether.

Ethyl Dithiocarbonates: Xanthates.

The xanthates have the composition of esters, but possess decided acid properties.   .

When boiling absolute alcohol is saturated with pure potassium hydroxide and carbon disulphide added gradually till it ceases to be dissolved, or the liquid becomes neutral, potassium xanthate is formed. On cooling, the xanthate crystallises in slender colourless prisms, which must be pressed between blotting-paper and dried in a vacuum. It is readily soluble in water and alcohol, but insoluble in ether. On exposure to air it suffers gradual decomposition.

On adding dilute sulphuric or hydrochloric acid to potassium xanthate, xanthic acid is liberated as a colourless, heavy, oily liquid, of peculiar and powerful odor and astringent bterit taste. It is very combustible. Xanthic acid reddens litmus, and ultimately bleaches it. At a very slight rise of temperature it undergoes decomposition into alcohol and carbon disulphide. Owing to this property the xanthates have been successfully used as a remedy for *Phylloxera*, which attacks the vine, and has been used against other noxious insects. The xanthate is mixed with earth, either alone or together with superphosphate, when it gradually undergoes decomposition with formation of carbon disulphide. Xanthic acid possesses powerful antiseptic propperties. Sodium xanthate is employed to effect the reduction of ortho-

nitrophenylpropiolic acid to indigo blue. When warmed with nitric acid, xanthic acid and xanthates evolve an odour suggesting ethyl nitrite. On distillation, the xanthates are decomposed with formation of carbon dioxide, carbon disulphide and hydrogen sulphide and a peculiar sulphuretted oil, while a sulphide and free carbon remain.

The most characteristic reaction of xanthic acid, and the one from which it derived its name, is that with copper. On adding copper sulphate to a neutral solution of a xanthate a brownish precipitate of cupric xanthate is first formed, which quickly changes to bright yellow flocks of cuprous xanthate. This substance is insoluble in water and in dilute acids, but is decomposed by strong acids. It is slightly soluble in alcohol and rather more so in carbon disulphide. It is not sensibly attacked by hydrogen sulphide, but is instantly decomposed by sodium sulphide. The formation of cuprous xanthate has been utilized for detecting carbon disulphide in illuminating gas, the gas being passed through alcoholic solution of potassium hydroxide, the excess of alkali neutralised by carbonic or tartaric acid, the insoluble salt removed by filtration, and the liquid treated with copper sulphate.

Xanthates may also be estimated by titration with a standard solution of iodine, or by oxidation with permanganate, and precipitation of the resultant sulphate by barium chloride.

Nitrous Ethers.—Two of these are of importance, owing to their use in medicine. Several other substances having formulas apparently identical with the derivatives of nitrous acid are, in reality, derivatives of nitric acid. Such are nitrobenzene and nitroethane. The true nitrous ethers (esters) are capable of saponification (see page 232) yielding a nitrite. Very minute amounts of nitrite can be detected by Griess' test, which consists in adding to the liquid to be tested a solution of sulphanilic acid, then a solution of a-naphthylamine, each dissolved in strong acetic acid. A nitrite will produce a red liquid. Only a very minute amount of the substance to be tested should be used and the solution should be allowed to stand for five minutes. It must be borne in mind, however, that nitrites are present in the air, water or even dust, hence error from these sources must be excluded. Commercial spirit of nitrous ether gives this reaction without saponification. One drop of the spirit in 50 c.c. of water is easily detected. It is possible that a slight hydrolysis occurs by which nitrous acid is formed.

Etyl Nitrite. Nitrous Ether.—This substance has been known in

an impure state for a long time. It may be obtained by passing the red vapours of nitrogen trioxide (evolved by acting on starch by nitric acid) into alcohol; by distilling potassium (or sodium) nitrite with alcohol and sulphuric acid; or by the direct action of nitric acid on alcohol. In the last case the nitric acid is reduced by a portion of the alcohol, and the nitrous acid so formed acts on the remainder to form ethyl nitrite. A considerable quantity of aldehyde results from the oxidation of the alcohol, so that the ether obtained by this process is largely contaminated. This reaction may be avoided in great measure by adding metallic copper to the contents of the retort.

Pure ethyl nitrite is a nearly colourless liquid of fragrant odour. It is soluble in all proportions in alcohol, but requires about fifty parts of water for solution. It boils at 18°, and has a sp. gr. of 0.947 at 15.5. It is liable to decompose on keeping, especially in presence of water. It gives many of the ordinary reactions of the nitrites. Thus, when mixed with a little dilute sulphuric acid, and poured on a strong aqueous solution of ferrous sulphate, it develops a dark brown ring; when dissolved in alcohol and treated with a few drops of dilute sulphuric or acetic acïd, it liberates iodine from potassium iodide, and therefore the mixture becomes blue on addition of starch.

Spirit of Nitrous Ether.—"Spirit of nitrous ether" (*Spiritus ætheris nitrosi*) is the present official name of a preparation consisting essentially of a solution of impure ethyl nitrite in rectified spirit. Spirit of nitrous ether is the modern representative of the old "sweet spirit of nitre" (*Spiritus nitri dulcis*, London Pharmacopœia, 1745), which was prepared by distilling together rectified spirit and nitric acid.

The characters of "spirit of nitrous ether" are thus described in the British Pharmacopœia of 1867: "Transparent and nearly colourless, with a very slight tinge of yellow, mobile, inflammable, of a peculiar penetrating apple-like odor, and sweetish, cooling, sharp taste. Sp. gr., 0.845. It effervesces feebly or not at all when shaken with a little "bicarbonate of soda." When agitated with solution of sulphate of iron and a few drops of sulphuric acid, it becomes a deep olive-brown or black. If it be agitated with twice its volume of saturated solution of calcium chloride in a closed tube, 2 per cent. of its original volume will separate in the form of nitrous ether, and rise to the surface of the mixture." In later reprints of the British Pharmacopœia of 1867 the words "an ethereal layer" are substituted for "nitrous ether" in the last sentence.

The spirit of nitrous ether of the German Pharmacopœia has a sp. gr. of 0.840 to 0.850; the United States Pharmacopœia requirement is 0.823 at 25°, and is described as containing not less than 4 per cent. of ethyl nitrite.

Spirit of nitrous ether is a liquid of very complex composition. Besides the ethyl nitrite, alcohol, and water which may be regarded as its normal constituents, it usually contains aldehyde, and probably paraldehyde and ethyl acetate and nitrate. After keeping, sensible quantities of free nitrous and acetic acids are developed, and other changes occur. In addition to the foregoing constituents, the occurrence of which is generally admitted; according to Eykman, spirit of nitrous ether is also liable to contain ethyl oxide (ether); ethyl formate and oxalate; cyanogen compounds; glyoxal; glyoxalic, oxalic, malic, and saccharic acids; to which list Allen suggested the addition, as a possible constituent, of *nitroethane*, a body isomeric with ethyl nitrite, but having a sp. gr. of 1.058 and boiling at 111° to 113°.

The tendency of spirit of nitrous ether and similar preparations to undergo gradual deterioration with destruction of the nitrous ether is a point of importance. The exact conditions are not thoroughly understood, but it is established that excess of water favors the destruction of the ester. Hence adulteration of sweet spirit of nitre with water, a common practice, not only dilutes the preparation, but greatly enhances the tendency of the nitrous ether to undergo decomposition. Allen found that a sample kept perfectly well for very many months when diluted, but a portion of the same mixed with one-third its volume of water contained no nitrous ether after an interval of 4 months. In these experiments the samples were kept in well-closed bottles. Imperfect closing of the bottle, exposure to light or to excessive temperature, will cause loss of so volatile a substance as this ester. On the other hand, a solution of the pure ester in nearly absolute alcohol was kept 7 years and still contained ethyl nitrite and mere traces of free acid.

Analysis of Spirit of Nitrous Ether.—The assay of spirit of nitrous ether is somewhat difficult, on account of the complex character of the preparation. The sp. gr., behaviour with sodium acid carbonate, and reaction with ferrous sulphate in presence of free acid are serviceable; but the test with solution of calcium chloride is worthless.

The following methods are the most satisfactory of many that have been devised:

Excess of water can be detected by the sp. gr. of the sample. The British Pharmacopœia spirit is 0.845 at 15.5°, but a slightly higher figure may be tolerated. If, however, it exceeds 0.853, excess of water is indicated. Commercial samples are sometimes adulterated so largely with water as to bring the sp. gr. to 0.910 or even higher; an inferior spirit of 0.900 sp. gr. being sold wholesale. A sp. gr. of 0.845 corresponds, according to the accepted alcohol tables to a content of 81.36 % by weight of absolute alcohol, or 151.78 % by volume of proof spirit. The extent to which a sample has been diluted with water may be found by multiplying the percentage of proof spirit (as found by the table) by the factor 657 ($= \frac{100}{151.78}$), when the product will be the percentage by volume of spirit of nitrous ether of standard contained in the sample. To find the percentage by measure of spirit of 0.850 sp. gr. originally present, the percentage of proof spirit in the sample should be multiplied by 0.673 ($= 100/148.8$).

The nitrous ether, though of higher sp. gr. than alcohol, is present in too small proportion to affect sensibly the estimation of water from sp. gr. The addition of water to sweet spirit of nitre is reprehensible, for it reduces the immediate strength and medicinal value of the preparation and renders it more liable to change.

Free acid will be indicated by the reaction with litmus paper, and by the effervescence occasioned on adding sodium carbonate to the sample. A notable proportion of free acid renders the spirit incompatible with potassium iodide, from which it liberates iodine. The proportion of acid may be ascertained by titration with standard alkali, but, as some samples contain both free acetic and free nitrous acid, it is sometimes of interest to determine them separately, which is done by P. MacEwan in the following manner: 10 c.c. measure of the sample is placed in a flask containing a drop of phenolphthalein solution, and two or three drops of methyl-orange solution are next added. A porcelain slab, spotted with drops of methyl-orange solution, is arranged in readiness. N/2 solution of sodium hydroxide is then rapidly added to the contents of the flask, and as soon as the red begins to fade, a drop of the liquid is removed by a glass rod and brought in contact with a spot of the methyl-orange on the plate. If the spot assumes a pink tint, the nitrous acid is not quite neutralised, in which case the addition of the alkali solution is continued, until, on retesting, a spot of methyl-orange is rendered only faintly pink. The volume of standard alkali used is then noted, and the titration continued until

the pink tint produced by the phenolphthalein denotes alkalinity. Each c.c. of the alkali first used represents 0.0235 grm. of nitrous acid, while each c.c. of the additional alkali requisite to produce the phenolphthalein reaction corresponds to 0.0300 grm. of acetic acid. The process is approximate only.

The United States Pharmacopœia requires that "when freshly prepared, or even after being kept for some time" the spirit of nitrous ether should not be acid to litmus. Even when quite old it should not effervesce with potassium hydrogen carbonate.

Aldehyde will be indicated by the brown produced on heating the sample with potassium hydroxide. A sample free from an excessive proportion of aldehyde, when treated at the ordinary temperature with half its volume of a dilute solution of potassium hydroxide, assumes a yellow colour which gradually deepens, but does not become brown in twelve hours.

The United States Pharmacopœia test for presence of aldehyde is:

If 10 c.c. of the spirit are mixed with 10 c.c. of potassium hydroxide of 3 % strength, the mixture will assume a yellow colour which should not turn decidedly brown within twelve hours.

Ethyl chloride and other **chlorinated bodies** may be detected by igniting a little of the sample in a porcelain basin and holding a beaker moistened with silver nitrate solution over the flame. If silver chloride be formed, the sample may be evaporated with sodium hydroxide and the chloride in the residue determined.

Total Ethyl Nitrite.—The following is a summary of the United States Pharmacopœia process for ascertaining the amount of ethyl nitrite in spirit of nitrous ether.

Shake 30 grm. of the sample with 0.5 grm. of potassium hydrogen carbonate, transfer the liquid to a tared flask marked at 100 c.c.; weigh accurately, add sufficient alcohol to make 100 c.c., and mix well. Put 10 c.c. of this mixture into a nitrometer, add 10 c.c. of potassium iodide solution (20 %) and 100 c.c. of normal sulphuric acid. Allow the volume of evolved gas to become constant—which will require about from 30 to 60 minutes—and note amount. Multiply this by 0.307 and divide by the weight of the sample taken. At the standard temperature used in the United States Pharmacopœia (25°) and at the usual standard pressure (760 mm.) the quotient will be the percentage of ethyl nitrite in the liquid. To correct for temperature deduct 1/3 of 1 % for each degree above the standard temperature, and add 1/3

of 1 % for each degree below that temperature. To correct for pressure add 4/30 for each mm. above the standard and deduct 4/30 for each mm. below the standard.

The following special requirement is also given by the United States Pharmacopœia: If a test-tube is half-filled with the spirit and put into a water-bath heated to 65° until it has acquired this temperature, the liquid should boil distinctly upon the addition of a few small pieces of broken glass.

Ethyl Nitrite may be detected by the brown produced by adding ferrous sulphate to an acidulated solution of the sample of spirit. Of various ways of making the test, Allen found the following the best: 10 c.c. of the spirit is mixed with 5 c.c. of a strong aqueous solution of ferrous sulphate. Pure, concentrated sulphuric acid should next be poured down the side of the test-tube in such a manner as to form a distinct stratum under the spirituous mixture. A brown zone will thereupon be produced at the junction of the two layers, the intensity of which is an indication of the strength of the sample in nitrous ether. With good samples, the colouration is increased and extended by causing the layers to become partially mixed, but with inferior specimens the brown colour is more or less destroyed by such treatment (see also page 241).

Methylated Spirit is said to be occasionally employed for the preparation of sweet spirit of nitre. The substitution may be detected by agitating 30 c.c. of the sample with 3 or 4 grm. of ignited potassium carbonate, treating 15 c.c. of the decanted dehydrated spirit in a small flask with 10 grm. of anhydrous calcium chloride, attaching a condenser, and heating the flask in boiling water till about 5 c.c. has passed over or scarcely any further distillate can be obtained. The operation proceeds slowly, but requires little attention and should be carried out thoroughly. The contents of the flask are next treated with 5 c.c. of water, and another 2 c.c. distilled. This second distillate is then oxidised by potassium dichromate and sulphuric acid, as described on page 236, and the product tested with silver nitrate. If the sample was free from methyl alcohol, the solution darkens, and often assumes transiently a purple tinge, but continues quite translucent; and the test-tube, after being rinsed out and filled with water, appears clean or nearly so. If the sample contains only 1% of methylic alcohol (= 10 to 20% of methylated spirit), the liquid turns first brown, then almost black and opaque, and a film of silver,

which is brown by transmitted light, is deposited on the tube. When the sample is methylated to the extent of 3 or 4 % the film is sufciently thick to form a brilliant mirror. The observations should be made by daylight.

Nitrates may be detected and determined by applying the phenoldisulphonic acid method (vol. 3, part 3, page 3, foot-note 3). The liquid should be saponified (see page 232) with sodium (or potassium) hydroxide, made up to a convenient volume, an aliquot part evaporated on the water-bath and treated with the reagent as directed at the reference given above.

Concentrated Spirit of Nitrous Ether.—For the convenience of pharmacists, some manufacturers prepare a mixture of nitrous ether and alcohol of definite strength, so that by adding a given volume to a given volume of alcohol, a liquid of official strength is obtained. A Philadelphia manufacturer has devised a plan of furnishing this concentrated form in sealed tubes of amber glass. These are well-cooled, the point broken, the contents promptly mixed with a pint of alcohol, and a little more than a pint of U. S. P. % spirit of nitrous ether obtained. The concentrated preparation usually contains about 16% of alcohol and 82 % of ethyl nitrite. Under ordinary circumstances, the assay of this will include estimation of the amount of ethyl nitrite and of the impurities to which that substance is liable. The simplest plan seems to be to add a measured volume of the sample to a known volume of pure alcohol and examine the liquid according to the methods already given for the commercial spirit.

W. A. Pearson (*Amer. J. Pharm.*, 1908, 80, 101) has devised a process for ascertaining the amount of alcohol in the concentrated preparation.

Ethyl Chloride. Hydrochloric Ether. "Sweet Spirit of Salt."

Ethyl chloride is a fragrant, volatile liquid, boiling at 12.2°, and burning when ignited with a smoky, green-edged flame, producing fumes of hydrochloric acid. Sp. gr., 0.911 to 0.916 at 8°. It is sparingly soluble in water, but readily so in alcohol, neither solution giving a precipitate with silver nitrate.

Ethyl chloride is used as an anesthetic and is now sold in sealed glass tubes which must be kept in a cool place. When the point of the tube is broken at ordinary temperatures, the liquid vapourizes at once, producing a readily inflammable vapor, much heavier than air.

The United States Pharmacopœia gives the following test for ethyl alcohol which may be present in the commercial chloride: 10 c.c. of the sample are agitated with 10 c.c. of cold water and the supernatant layer of ethyl chloride allowed to evaporate spontaneously. A few drops of dilute sulphuric acid and a few drops of potassium dichromate solution are then added and the mixture boiled. Alcohol will be indicated by an odor of aldehyde and a greenish or purplish tint in the liquid.

Ethyl chloride, evaporated from clean, bibulous paper, should leave no unpleasant odour.

Ethylidene Chloride, Chlorinated Ethyl Chloride, or β-Dichlorethane, $CH_3.CHCl_2$. This is now prepared in a pure state by the action of chlorine on ethyl chloride, or by distilling aldehyde with phosphorus pentachloride. Ethylidene chloride possesses anæsthetic properties. The isomer, ethylene dichloride, Dutch liquid, produces severe convulsions when its vapour is inhaled. Ethylidene chloride is distinguished by not reacting with potassium, whereas Dutch liquid is violently acted on, forming a porous mass and evolving hydrogen and chlorethylene, C_2H_3Cl, the latter being a gas of alliaceous odour. The same gas is produced when Dutch liquid is heated with alcoholic solution of potassium hydroxide, while ethylidene chloride is unaffected. The b. p. and sp. gr. also distinguish Dutch liquid from its isomer. From chloroform, ethylidene chloride is distinguished by its sp. gr., b. p., and negative reaction with Hofmann's test. (See p. 274).

Ethyl Bromide. Hydrobromic ether.

This has been employed in medicine as an anæsthetic. It boils at 38.4°, and has a sp. gr. of 1.473 at 0°. It burns with difficulty, giving a bright green but smokeless flame, and forming fumes of hydrobromic acid. The b. p. and smokeless flame distinguish it from ethyl chloride.

Ethyl bromide is liable to contain an admixture of ethyl ether, which reduces the sp. gr., Some samples are contaminated with an acid impurity that has an extremely unpleasant odour, and is less volatile than ethyl bromide. Such specimens are unfit for use. For general tests see under Ethyl Chloride.

Ethyl carbamate.

Carbamic acid is amidocarbonic acid It can be obtained by the action of ethyl alcohol upon urea. It forms colourless, odourless

crystals having a sharp, somewhat cooling taste. It is soluble in less than its weight of water and alcohol, in about an equal weight of ether, in 1.3 parts of chloroform and 3 parts of glycerol. These proportions are for a temperature of 25°. It melts between 47.5° and 50° and at a higher temperature it is decomposed, burning without leaving an appreciable residue.

The following tests for purity are condensed from the United States Pharmacopœia. Ethyl carbamate mixed with 5 times its weight of sulphuric acid and heated, evolves carbon dioxide and leaves alcohol and ammonium hydrogen sulphate in the liquid.

Ethyl carbamate heated with concentrated potassium hydroxide solution yields ammonium hydroxide.

A solution of ethyl carbamate in water, mixed with sodium carbonate and a little iodine and warmed, will on cooling deposit iodoform.

Strong solution of ethyl carbamate in water should give no precipitate with nitric acid, mercuric nitrate or oxalic acid.

The assay of ethyl carbamate can be carried out by the ordinary Kjeldahl method. One hundred parts of the carbamate will yield 51.7 parts of alcohol and 19.1 parts of ammonia (NH_3).

Amyl Acetate. Pentyl acetate.

Amyl acetate is obtained by distilling amyl alcohols with an acetate and sulphuric acid. As 8 isomeric forms of amyl alcohol exist, the properties of the acetate will differ considerably in different preparations. The isoamyl form is generally present in dominant amount. It is a colourless liquid, very slightly soluble in water, but soluble in alcohol, ether and amyl alcohols. It boils at 148° and has a sp. gr. of 0.8963 at 0°.

Amyl acetate may be determined by the general method on page 237. From *alcohol* it may be separated by agitating the liquid with an equal measure of saturated solution of calcium chloride which dissolves the alcohol only.

Amyl alcohols may be separated and determined approximately by treating the sample in a graduated tube with a mixture of equal volumes of glacial acetic acid and water.

This dissolves amyl alcohols, but leaves amyl acetate insoluble (together with any amyl valerate or pelargonate which may be present). By first separating the ethyl alcohol by salt water, or petroleum spirit, this method may be applied to the examination of the essence of jargonelle pear.

Amyl Nitrite.

Amyl nitrite is prepared by processes similar to those employed for obtaining ethyl nitrite, amyl alcohols being substituted for alcohol. To obtain a product fit for medicinal use, the amyl alcohols should be purified, and have a b. p. of 129° to 132°.

By passing vapour of nitrous acid (best prepared by the reaction of nitric acid on arsenous oxide) into this alcohol, a nearly pure nitrite is obtained. After washing the product with water and solution of sodium carbonate the oily liquid is rectified, the fraction passing over between 90° and 100° being retained. By carefully re-fractionating the distillate with a dephlegmator (page 21) a better product may be obtained, but it must be again washed with sodium carbonate to separate traces of acid produced by decomposition of the ether during redistillation.

Isoamyl nitrite has a sp. gr. of 0.902, and boils at from 94° to 95°. It has a yellowish colour, penetrating apple-like odor, pungent aromatic taste, and produces a very powerful effect on the system when its vapour is inhaled. It burns, when ignited, with a fawn-coloured smoky flame.

Amyl nitrite is insoluble in water, but soluble in alcohol in all proportions. It also dissolves in amyl and methyl alcohols, in glacial acetic acid, and is miscible in all proportions with ether, chloroform, carbon disulph de, benzene, petroleum spirit, and oils.

In contact with the air, and apparently more readily under the influence of light, amyl nitrite develops an acid reaction owing to partial decomposition. Probably this change occurs more readily in presence of moisture.

Concentrated sulphuric acid attacks amyl nitrite with great energy, red fumes being evolved, and a black, foul-smelling liquid formed. Occasionally the mixture inflames.

A characteristic test for amyl nitrite is the formation of potassium valerate when the liquid is dropped on melted potassium hydroxide. When gently warmed with excess of an aqueous solution of potassium hydroxide, potassium nitrite is formed, and a stratum of amyl alcohol floats on the surface of the liquid. The change occurs more readily by using the alcoholic solution and subsequently adding water to cause the separation of the amyl alcohol. On removing the aqueous liquid, acidulating it with acetic acid, and adding potassium iodide, the nitrite will occasion an abundant liberation of iodine. Griess' test can also be used (page 241).

When amyl nitrite is distilled slowly with methyl alcohol it is com-

pletely decomposed, with formation of amyl alcohol and methyl nitrite. Ethyl alcohol causes a less complete change, but it is evident that an alcoholic solution of amyl nitrite would be liable to undergo decomposition.

The United States Pharmacopœia defines Amyl nitrite to be a liquid containing about 80% of the ester (principally isoamyl nitrite) when assayed by the specified method (see below). The sp. gr. of the official form ranges from 0.865 to 0.875 at 25°.

The following qualitative tests are recommended:

5 c.c. of the sample are shaken a few times with 10 c.c. of water, 1 c.c. of normal potassium hydroxide, and a drop of phenolphthalein solution. The colour of the liquid should not be entirely discharged,

A mixture of 1.5 c.c. of N/10 silver nitrate and 1.5 c.c. of alcohol with a few drops of ammonium hydroxide should not become brown or black if mixed with 1 c.c. of the nitrite and gently heated. This is the method suggested for detecting aldehyde which would reduce the silver salt.

Commercial Amyl Nitrite.—The amyl nitrite commonly met with is sometimes far from pure, being liable to contain ethyl and amyl alcohols, amyl nitrate, butyl and hexyl nitrites, nitropentane, valeric aldehyde, water, and other bodies. Amyl nitrite prepared as on page 250, will contain most of these in only very insignificant proportion, but they will be present if impure fusel oil is substituted for purified amyl alcohol, or if the latter is converted by treatment with nitric acid instead of nitrous acid, as is done by some manufacturers.

The following table shows the composition, densities, and b. p. of of some substances likely to be present in commercial amyl nitrite:

Name.	Sp. gr.	B. p.
Nitropentane,	0.877	150–160
Amyl nitrite,	0.902 at 0° C.	96
Amyl nitrate,	1.000 at 7°	148
Amyl alcohol,	0.814 at 15°	128–131
Valeraldehyde,	0.8057 at 17°	92.5

Valeraldehyde is not likely to be removed by fractional distillation, though the other impurities can be more or less eliminated. Admixture of valeric aldehyde or amyl alcohol reduces the sp. gr. while amyl

nitrate increases it. The latter has a comparatively high b. p., hence an instructive examination of amyl nitrite can be made by distilling the sample with a dephlegmator and noting the volumes, sp. gr. and odours of fractions collected at different temperatures. A fairly pure article, when fractionally distilled, will yield at least 80% of its original measure between 90° and 100°, and should leave no considerable residue at the latter temperature. Some specimens have been found to boil at temperatures ranging from 70° to 180°, and occasionally to leave a residue at 220°. As a rule, incomplete distillation at 100° is due chiefly to the presence of amyl alcohol, some of which may be formed by partial decomposition of the nitrite during distillation. Hence commercial amyl nitrite of good quality may leave a residue of 5 to 10% at 100°.

A further examination of the nature of the 90° to 100° fraction might be made by gently heating it for some time with methyl alcohol in a flask furnished with an inverted condenser. On subsequent distillation, the fraction passing over between 90° and 100° will consist mainly of the *valeraldehyde* of the original sample, the amyl nitrite having been converted into amyl alcohol and the very volatile methyl nitrite.

Nitropentane, a body isomeric with amyl nitrite, appears to be present in most commercial specimens of the latter, and sometimes in notable quantity. It may be detected by subjecting the fraction distilling between 140° and 170° to the action of nascent hydrogen when amylamine, will be formed, and may be recognised by the alkaline character of the distillate obtained on boiling with potassium hydroxide. Nitropentane may also be detected by dissolving the 140° to 170° fraction in solution of potassium hydroxide, adding a little sodium nitrite, and then dilute sulphuric acid very cautiously, when a blood-red colouration will be produced, which disappears when the solution becomes acid. Pentyl nitrolic acid is produced and may be extracted by agitation with ether. Probably the test might be applied by warming the original sample with alcoholic potassium hydroxide and cautiously adding dilute sulphuric acid.

Amyl Nitrate, if present, will be contained in the last fractions obtained on distilling a sample of amyl nitrite.

Valeric Aldehyde, may be detected by treating the sample with three measures of a mixture in equal parts of strong ammonium hydroxide and absolute alcohol, then adding a few drops of silver-

nitrate solution and warming gently, when a dark brown will be produced if valeric aldehyde be present.

Butyl and hexyl compounds may be detected by saponifying the sample with sodium hydroxide and examining the amyl acohol layer for butyl and hexyl alcohols by distillation.

Free acid may be detected and estimated as in spirit of nitrous ether after dissolving the sample in rectified spirit.

Water increases the sp. gr. of the preparation, and renders it turbid in melting ice. The presence of water increases the tendency to decomposition.

Hydrogen cyanide, occasionally present as a by-product, may be recognised by largely diluting the sample with alcohol and adding silver nitrate, when white curdy silver cyanide will be precipitated.

The United States Pharmacopœia process for assay of amyl nitrite is the same as that for ethyl nitrite (page 245) except that 3 c.c. of the sample are shaken with potassium hydrogen carbonate and then decanted and weighed in the tared 100 c.c. flask. The reading of the volume of gas multiplied by 4.8 and divided by the weight of sample taken gives the percentage of nitrite present at 25° and 760 mm. Corrections for difference of temperature and pressure are made according to the rule for assay of ethyl nitrite.

ALDEHYDES.

The aldehydes are a series of compounds intermediate in composition between the alcohols and their corresponding acids.

Aldehydes result from the treatment of the corresponding alcohols by oxidising agents of moderate power, such as dilute nitric acid or dilute chromic acid mixture used cautiously. They are also formed by distilling a mixture of the sodium or calcium salt of the corresponding acid with calcium formate.

Aldehydes may also be obtained by the action of nascent hydrogen on the chlorides of the corresponding acid radicles, and by various other reactions.

When pure, the aldehydes may apparently be preserved without change, but the presence of mere traces of impurity (e. g., mineral acids), tends to cause their gradual conversion into polymers or condensation-products, in the latter case water being simultaneously eliminated.

By oxidation, the aldehydes are very readily converted into the corresponding acids. Hence, they are powerful reducing agents, precipitating metallic silver from the ammonio-nitrate and decolourising permanganates.

By the action of nascent hydrogen (sodium amalgam), the aldehydes are reduced to the corresponding alcohols, but the fixation of hydrogen is often attended with condensation, and consequent coformation of a higher diatomic alcohol.

When heated with solutions of alkalies, the aldehydes are mostly converted into resinous bodies. By fusion with potassium hydroxide aldehydes are converted into the potassium salts of the corresponding acids, hydrogen being simultaneously evolved; in some cases this acts on another portion of the aldehyde and converts it into the corresponding alcohol.

Many of the aldehydes form compounds with water, hydrogen chloride and other bodies, but the products are very unstable.

The aldehydes readily combine with ammonia (NH_3), the products first formed often undergoing molecular condensation more or less rapidly. The ammonia compounds of the aldehydes of the acetic series are not liable to this change, and are stable crystalline bodies which liberate the original aldehyde on treatment with dilute sulphuric acid.

Many aldehydes and allied bodies (ketones), have the property of forming stable crystalline compounds with acid sulphites. The sodium compound is readily obtained on treating the aldehyde or its aqueous solution with excess of a saturated cold solution of sodium hydrogen sulphite, when the compound separates in crystals which are soluble in water or alcohol, but insoluble in a strong solution of the reagent. From this compound the aldehyde may be regenerated by treatment with dilute sulphuric acid (or sodium carbonate), or sometimes by simply warming the aqueous solution. Aldehydes of the acetic series (as also chloral) reduce hot Fehling's solution, but aldehydes of the aromatic series do not.

Most substances of the aldehyde class give colouration with an acid solution of rosaniline previously mixed with sufficient sodium sulphite almost to decolourise it. (See page 257.) Examined in this way, acetaldehyde, paraldehyde, and propionaldehyde give an intense red-violet colouration. Chloral gives at once a fine colour, but chloral hydrate gives no reaction. Acrolein and butyl chloral produce a violet

colour on shaking. Furfural and benzaldehyde give the colour more readily. Salicylic and cuminic aldehydes react well after some agitation. Cinnamic aldehyde and furfur-acrolein give at first an intense yellow colour, soon changing to violet-red. Acetone readily reacts on shaking, but acetophenone and benzophenone have no action. Methyl and ethyl alcohols give a faint violet colour on shaking, propylic and isopropylic alcohols a scarcely perceptible reaction, while with their higher homologues, and phenols, glycols, quinine, sugars, and formic acid, no colour is obtained.

A mere trace of most bodies of the aldehyde class produces a fine scarlet colour with a solution of phenol in excess of sulphuric acid, the colour changing to a dark red on warming the mixture.

A delicate test for aldehydes is the violet-red colour they give with diazobenzene-sulphonic acid in presence of free alkali. 1 part of freshly-prepared diazobenzene-sulphonic acid is dissolved in 60 parts of cold water rendered alkaline by sodium hydroxide. To this solution is added the liquid to be tested (previously mixed with dilute solution of sodium hydroxide) together with a little sodium amalgam. If an aldehyde be present, an intense violet-red is produced, either immediately or within 20 minutes. The colour is destroyed by long exposure to the air, and is changed by the addition of an acid.

The reaction is readily yielded by a solution containing 1 part in 3,000 of benzaldehyde (oil of bitter almonds), and has been obtained with acetic, valeric, and œnanthic aldehydes, as also with furfural and glyoxal. Chloral and benzoin do not give the reaction. Acetone and ethyl aceto-acetate give a red colour, but without the violet tint characteristic of an aldehyde. The reaction is not produced by phenol, resorcinol, or pyrocatechol (if care be taken to have excess of alkali present), but is given by dextrose. It is said to be more delicate than that with rosaniline reduced with sulphurous acid; but the reaction is more especially suitable for the detection of aldehydes which are permanent in alkaline solutions.

E. Fischer recommends the employment of phenylhydrazine hydrochloride as a reagent for detecting bodies of the aldehyde class.

The ammoniated silver solution described on page 265 is a general test for aldehydes.

Acrolein, valeral, furfural and the **essential oils** of bitter almonds, cinnamon, cloves, cumin, and meadow-sweet have the consti-

tution and characters of aldehydes. All these form crystalline compounds with acid sulphites.

Acetone and acetal are bodies allied to the aldehydes, and **chloral** is a trichloraldehyde.

According to Ripper, (*Monatsh. d. Chem.*, 1900, 21, 1079), any aldehyde soluble in water or in very dilute alcohol can be assayed as follows: (See page 266.)

A convenient amount of the solution of the aldehyde is mixed with twice its bulk of a solution of acid potassium sulphite (containing 12 grm. in 1000 c.c.) The mixture is allowed to stand for 15 minutes, and the unprecipitated sulphite determined by titration with iodine. The sulphite solution must, of course, be valued by a similar titration.

Formic Aldehyde. Formaldehyde. Methaldehyde.

This body is produced by the limited oxidation of methyl alcohol. Its formation is the first stage in the production of carbohydrates in plants by the decomposition of carbon dioxide, hydrogen dioxide being produced at the same time. It presents a general resemblance to ordinary or acetic aldehyde, but it is polymerised with exteme readiness. It is gaseous at the ordinary temperature, a polymer, paraformaldehyde, is a white insoluble body, subliming at the temperature of boiling water, and suffering depolymerisation at a higher temperature, or when heated to 140° with excess of water in a sealed tube.

Ordinary formaldehyde undergoes slow oxidation in the air, forming formic acid. It is rapidly oxidised by the more powerful oxidising agents. When its aqueous solution is mixed with solid potassium permanganate, a violent reaction occurs, some of the aldehyde is oxidised to carbon dioxid and water, and another portion escapes in the form of vapor. The gases eliminated are often combustible. This reaction has been utilised for obtaining formaldehyde vapour in disins fecting large inclosed spaces, for which it is especially applicable. The danger of fire from the spontaneous ignition of the evolved gases must be kept in mind.

Formaldehyde reacts with ammonium hydroxide forming a substitution amine (see page 263) When heated with sodium hydroxide for some time on the water-bath, formaldehyde forms sodium formate and methyl alcohol.

The solid polymer is now sold under the name "paraform" for disinfecting purposes. This material begins to sublime at 100° and melts between 153° and 172°, producing gaseous formaldehyde.

The United States Pharmacopœia solution of formaldehyde (*Liquor formaldehydi*) istrequired to contain not less than 37% by weight of the aldehyde. The sp. gr. of this solution ranges from 1.075 to 1.081 at 25°. It should not contain more than 0.2% of free acid, calculated as formic.

Formaldehyde has acquired great importance within the last few years on account of its employment as a disinfectant and food preservative. The literature concerning it is extensive, much of it relates to the detection of the substance in food, especially milk. It is principally sold as a 40% (by volume) solution in water, under the name "formalin." Formaldehyde forms compounds with many albuminous and gelatinous substances, often rendering them very insoluble. A few drops of formalin added to a solution of gelatin cause the liquid to set to a mass which cannot be melted when held in a flame. The compounds obtained in this manner retain to some extent the properties of formaldehyde.

When solutions of formaldehyde are boiled, a considerable portion of the substance passes over with the steam, but if the distillate be transferred to a dish on the steam-bath and evaporated, much of the substance will remain as a white solid—the polymeric modification.

Many tests for formaldehyde have been published. Some of these are general tests for the aldehydes (see p. 253). The following are mostly of this character, but they are especially employed for the detection of formaldehyde.

Fuchsin Test.—This is performed with Schiff's reagent, for the preparation of which Allen suggested the following: 40 c.c. of a 5% solution of magenta (fuchsin) are mixed with 250 c.c. of water, 10 c.c. of sodium acid sulphite solution of 1.375 sp. gr., and then 10 c.c. of pure sulphuric acid. The mixture is allowed to stand for some time, when it will become colourless. The addition of a solution of formaldehyde restores the red of the dye, but a colour resembling that caused by formaldehyde may be obtained by blowing air through the reagent, by contact with aerated water or even by warming.

Betanaphthol Test (Mulliken, *Ident. Pure Org. Comp.*, vol. 1).— The solution to be tested is mixed with 3 c.c. of dilute alcohol (1 : 2), 0.005 grm. betanaphthol and 3 to 5 drops of hydrochloric acid and boiled for few minutes. Any precipitate is collected on a filter washed with dilute alcohol of the same strength as that first used, dissolved in a small amount of alcohol by the aid of heat, the liquid cooled, the

crystals that separate collected on a filter washed with 1 c.c. of strong alcohol dried on a porous tile in a warm place and the m. p. determined. The precipitate when formaldehyde is present is methylene-dibetanaphthol $(CH_2)(C_{10}H_6HO)_2)$, melting at 189°.

Salicylic acid Test.—A small amount of salicylic acid is dissolved in a few c.c. of strong sulphuric acid. When this mixture is warmed gently with formaldehyde a red liquid is produced.

Phenylhydrazine-nitroprusside Test.—Rimini's test. A small amount of phenylhydrazine hydrochloride is added to the solution to be tested, then a drop of dilute solution of sodium nitroprusside and a few drops of sodium hydroxide solution. The liquid becomes deep blue if formaldehyde is present. The nitroprusside solution should be freshly prepared. With milk containing formaldehyde this test produces a greyish-green.

Phloroglucol Test.—A small amount of a freshly prepared (about 1%) solution of phloroglucol in water is mixed with an equal measure of a 25% solution of sodium hydroxide, and the solution to be tested added. The liquid becomes rose-red. It is best to introduce the liquid to be tested by means of a pipette, so as to underlay the reagent solution. The color then appears at the junction of the liquids.

Hydrochloric Acid Test.—1 c.c. of hydrochloric acid containing a little iron is added to 4 c.c. of the liquid to be tested and the mixture heated to boiling. If formaldehyde is present the liquid will become red. The test does not work well if much formaldehyde is present. If the liquid becomes yellow on heating, some of the original solution should be considerably diluted and the test repeated. Many samples of commercial hydrochloric acid contain enough iron to give the reaction. Pure acid may be made applicable by adding ferric chloride in the proportion of 0.025 grm. to 100 c.c.

Bonnet's Test (*J. Amer. Chem. Soc.*, 1905, **27**, 601).—A small amount of morphine is placed on a watch-glass, a drop or two of sulphuric acid added, the mass stirred with a glass rod, and the watch-glass floated on the surface of the liquid to be tested. The whole is then covered with a glass or porcelain cover and allowed to remain for at least thirty minutes. If formaldehyde is present the mixture in the dish will become dark.

This test has the advantage that the reaction can only be due to a volatile ingredient, and the interfering or misleading reactions of substances in complex organic mixtures, such as milk, are avoided.

The dimethylaniline test, suggested by Trillat, and described in the previous edition of this work, has been shown by B. M. Pilhashy (*J. Amer. Chem. Soc.*, 1899, 21, 134) to be untrustworthy, the colour reaction being due to the reagents.

Resorcinol Test (Lebbin).—A few c.c. of the liquid to be tested are boiled with 0.05 grm. of resorcinol, to which half or an equal volume of a 50% solution of sodium hydroxide is added. If formaldehyde is present, the yellow solution changes to a fine red. Analogous compounds showing the usual reactions characteristic of alhedydes fail to give this colouration.

Phenol Test (Hehner).—If to an aqueous solution of formaldehyde one drop of a dilute aqueous solution of phenol be added, and the mixture be poured upon some strong sulphuric acid in a test-tube, a bright crimson zone appears at the point of contact of the two liquids. The reaction must be carried out as described. A trace only of phenol must be used, and it must be first mixed with the solution to be tested before adding to the sulphuric acid.

Milk Test (Hehner).—Milk containing formaldehyde produces with strong sulphuric acid a purple-violet liquid. The test is best applied by underlaying the milk with the acid, when the colour is seen just below the line of junction. The acid must contain a trace of iron, which can be easily secured by adding a drop or two of ferric chloride to 5 c.c. of the pure acid. Good results are obtained by putting a few crystals of potassium sulphate into the milk before underlaying it with the acid.

Shrewsbury-Knapp Test (*Analyst*, 1909, 34, 12).—H. S. Shrewsbury and A. W. Knapp find that a reagent prepared by mixing 1.6 c.c. of N/1 nitric acid and 100 c.c. of concentrated hydrochloric acid produces violet with very minute quantities of formaldehyde in milk. The reagent must be freshly prepared. 10 c.c. of it are added to 5 c.c. of the milk in a test-tube, the tube placed in a water-bath at a constant temperature of 50° for 10 minutes, then cooled rapidly to 15°. Quantitative estimations can be made by comparing the color with that produced by milk containing known amounts of formaldehyde. If the depth of colour produced by the sample is deeper than produced by 6 parts per million of formaldehyde, it is best to dilute the sample and make a new test.

The observers found that the most delicate quantitative reactions were obtained with milks containing from 0.2 to 6.0 parts per million.

Several other oxidizing agents were tried by them, but were found to be inferior to nitric acid. They consider that the dyes occasionally used in adulterating milk will not interfere with the test.

C. H. LaWall (*Proc. Pa. Pharm. Assn.*, 1905, 200) found that vanillin simulates formaldehyde in the sulphuric acid contact test, the resorcinol test, and the phenol-sulphuric acid test. As vanillin is often used in association with foods that are likely to be preserved with formaldehyde, care must be taken not to overlook this simulation. Lawall found that the phenylhydrazin test does not react with vanillin.

QUANTITATIVE METHODS.

The tests for formaldehyde are best applied to pure solutions in water. As the substance is readily volatile with steam, some of it can usually be obtained in satisfactory form by simple distillation, but the amount that passes over is uncetain and it is often impossible to obtain a distillate containing all the formaldehyde. The estimation of the small quantities employed for preserving milk is especially attended with great difficulty. The preliminary isolation of the preservative by distilling the milk is open to objection, but the experiments made by Leonard and Smith (*Analyst*, 1897, 22, 5) show that rough indications of the amount of formaldehyde present can be obtained with certain precautions: (1) The distillate from fresh milk exerts no appreciable reducing action on alkaline permanganate, but milk 3 or 4 days old yields a distillate having marked reducing properties. (2) The separation of formaldehyde from milk is facilitated by acidifying the liquid, with sulphuric acid and blowing live steam through it. Under these conditions the first 20 c.c. of distillate from 100 c.c. of milk will contain about 1/3 and the first 40 c.c. about 1/2 of the total amount of formaldehyde present. (3) The fact that the distillate from milk does not contain the whole of the formaldehyde present is to a great extent explained by the behaviour of solutions of formaldehyde on distillation, and is only partly due to any specific action of the preservative on the constituents of milk.

Several methods of assay have been suggested, some of which are described below. According to R. H. Williams (*J. Amer. Chem. Soc.*, 1905, 27, 596), the iodine method is best adapted to pure dilute solutions; the potassium cyanide method to impure dilute solutions; the hydrogen peroxide method, directed by the United States Pharmacopoeia, to strong impure solutions.

Iodiometric Method (Romijn, *Zeit. Anal. Chem.*, 1897, **36**, 18.—10 c.c. of the solution to be tested are mixed with 25 c.c. of decinormal iodine and sodium-hydroxide solution added, drop by drop, until the liquid becomes clear yellow. After ten minutes hydrochloric acid is added and the free iodine is titrated with decinormal sodium thiosulphate. Two atoms of iodine are equivalent to 1 molecule of formaldehyde. This method is suitable for the accurate determination of formaldehyde alone, but does not give good results in the presence of other aldehydes and ketones.

Potassium Cyanide Method.—This is based upon the fact that formaldehyde combines with potassium cyanide. The addition-product reduces silver nitrate in the cold, but if the silver nitrate be acidified with nitric acid before the addition of the aldehyde mixture, no precipitate results if the aldehyde in the latter be in excess. If, on the other hand, the cyanide is in excess, one molecule of potassium cyanide is left in combination with one molecule of the formaldehyde, while the excess precipitates silver cyanide from the silver nitrate solution.

10 c.c. of decinormal silver nitrate, acidified with nitric acid, are mixed with 10 c.c. of potassium-cyanide solution (prepared by dissolving 3.1 grms. of the 96% salt in 500 c.c. of water), the whole diluted to 500 c.c., filtered, and 25 c.c. of the filtrate titrated by Volhard's method. The difference between this blank result and that obtained by titrating the filtrate after the addition of the aldehyde solution gives the amount of decinormal sulphocyanate corresponding to the silver not precipitated by the excess of potassium cyanide. From this the amount of formaldehyde can be calculated. Results by this method are said to be correct, even in the presence of acetaldehyde, if titrated immediately after shaking.

Hydrogen Peroxide Method.—Blank and Finkenbeiner first described this method (*Ber.*, 1893, **31**, 2979). It was further studied by Fresenius and Grünhut (*Zeit. anal. Chem.*, 1905, **44**, 13). R. H. Williams (*J. Amer. Chem. Soc.*, 1905, **27**, 596) and J. K. Haywood and B. H. Smith (*J. Amer. Chem. Soc.*, 1905, **27**, 1183). The last-mentioned workers give the process substantially as given by the U. S. Pharmacopœia, but advise taking the sp. gr. of the formaldehyde solution and calculating the weight of a definite volume while the U. S. P. directs the weighing of the amount of solution used. The U. S. P. process is here given.

3 c.c. of the solution are weighed accurately in a well-stoppered

Erlenmeyer flask, 50 c.c. of normal sodium hydroxide are added, the liquids mixed and 50 c.c. solution of hydrogen peroxide added "immediately, but slowly, through a small funnel." Previous to addition, the hydrogen peroxide should have been exactly neutralised by normal sodium hydroxide, using litmus as an indicator. The addition of the hydrogen peroxide will cause foaming. When this has ceased, rinse the funnel and the sides of the flask with distilled water, allow to stand for 30 minutes and titrate to neutrality with normal sulphuric acid. The number of c.c. of alkali consumed by the formic acid produced by the oxidation, multiplied by 2.979 and divided by the weight of solution taken will give the percentage by weight of absolute formaldehyde present. The hydrogen peroxide should be the standard, 10 volumes (about 3%), solution.

Ammonia Method.—A. G. Craig (*J. Amer. Chem. Soc.* 1901, **23,** 642) finds that the reaction between ammonia and formaldehyde (see page 263) can be used according to the following manipulation:

Several stout bottles holding about 100 c.c. and provided with good rubber stoppers are selected, and a vessel that will allow them to be submerged while standing upright. In each of the bottles is placed 25 c.c. of normal ammonium hydroxide (it is not necessary that this should be exactly of that strength). In some of the bottles accurately measured amounts of the solution to be tested are placed, using a quantity containing about 0.5 grm. of the aldehyde.

The bottles are securely corked, tied down, placed in the heating vessel, submerged with cold water and the latter raised to the b. p. Water must be added cautiously from time to time, keeping the bottles submerged and upright. After being in the bath for 1 hour, they are removed, cooled, opened, a little methyl orange added to each and then each titrated with normal sulphuric acid until the *first red* tint appears. The difference between the amount of acid required for the blanks and that for the bottles in which the formaldehyde solution was placed is the equivalent of the ammonia used by the latter. One c.c. of normal ammonium hydroxide is taken up, 0.0601 of by formaldehyde.

Gasometric Method.—G. B. Frankforter and R. West (*J. Amer. Chem. Soc.* 1905, **27,** 714) find that estimation of formaldehyde may be made by measuring the gas evolved when hydrogen dioxide is added to a mixture of formaldehyde and potassium hydroxide. The reaction is

$$2CH_2O + 2KHO + H_2O_2 = 2KCHO_2 + 2H_2O + H_2.$$
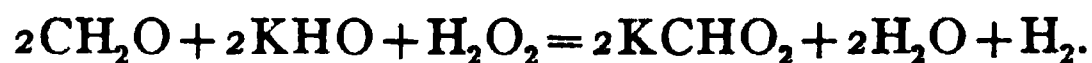
Hexamethylene-tetramine. Hexamethylene-amine. $(CH_2)_6N_4$.
—This body is a substitution amine (tetramine), but as it is a direct product from formaldehyde and owes its importance entirely to its therapeutic relation to that substance, it will be described here. It is produced by the action of formaldehyde on ammonia, the equation reaction being

$$6CH_2O + 4NH_3 = (CH_2)_6N_4 + 6H_2O.$$
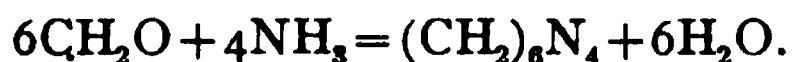
The compound forms colourless, odourless, glistening crystals, soluble in about 1.5 parts of water, both cold and boiling, 10 parts of alcohol, and sparingly in ether. The solution in water is alkaline to litmus; the tannin and mercuric chloride give precipitates with it. The solid is volatilised and partially decomposed by heating. Strong sulphuric acid converts it into ammonium sulphate and formaldehyde. A mixture of it with a little salicylic acid becomes red on warming with sulphuric acid.

This substance is used especially as a means of introducing formaldehyde into the genito-urinary tract, in the treatment of suppurative diseases of the kidneys, bladder and urethra. It is apparently partly hydrolysed in the system, reproducing formaldehyde and ammonium hydroxide. The former is excreted with the urine. The compound is now sold under many proprietary names, among which are "urotropin," "formin," "cystogen."

In addition to the tests above given , an assay of a sample could readily be made, by the Kjeldahl-Gunning method. The pure substance contains 40 per cent. of nitrogen.

The frequent use of hexamethylene amine in proprietary medicines and nostrums renders it necessary to note some processes especially adapted to detecting it in such articles. Horton (*Ber.*, 1888, **21**, 2000) found that bromine forms a brick-red precipitate, having the composition $C_6H_{12}N_4Br_4$, which on drying becomes yellow and is converted into the dibromide, $C_6H_{12}N_4Br_2$. Dobriner (*Zeit. anal. Chem.*, 1897, **36**, 44) found that mercuric chloride produces precipitates which differ with the ratio of the reagent to the hexamethylene-amine, when the latter is in excess, the precipitate consists of monoclinic prisms containing $2C_6H_{12}N_4 + 3HgCl_2$, but when the reagent is in excess the compound $C_6H_{12}N_4 + 6HgCl_2$ is formed.

The bromine and mercuric chloride compounds may be further tested for identification. The dibromide melts slightly below 200°.

The amounts of bromine and nitrogen present may be ascertained by the usual methods. In the case of the mercuric chloride compound, which should be obtained by using excess of the reagent, the proportions of mercury and chlorine will be important data.

Separation of Formaldehyde and Acetaldehyde.—Mulliken and Scudder have described the following method:

A round-bottom flask is connected with a long spiral reverse condenser, through which water at from 45° to 50° is passed. The upper part of the spiral is connected tightly with a tube which turns and is connected to a descending spiral condenser which is surrounded with ice and salt and is tightly connected to a flask, also immersed in a freezing mixture. All the condensing apparatus should be of glass. A suitable amount of the aldehyde mixture is placed in the distilling flask and gently boiled for two hours. Only traces of formaldehyde pass into the receiver, but the acetaldehyde distils over. The liquid to be distilled should not contain over 1 per cent. of formaldehyde.

Acetaldehyde. Acetic Aldehyde. Ethyl Aldehyde.

This is the body from which the class of aldehydes derived its name, and when the term "aldehyde" is used without qualification acetic aldehyde is understood.

Aldehyde results from the destructive distillation of various organic compounds and from the limited oxidation ·of alcohol, as by dilute chromic acid, or by air in presence of platinum black. In practice it is prepared by distilling together alcohol, sulphuric acid, and manganese dioxide.

It is a colourless, mobile liquid, with a pungent, suffocating odor. The disagreeable odour is much stronger in the crude substance. Its sp. gr. is 0.790 and it boils at 22°. It does not redden litmus, but on exposure to air, oxidises slightly to acetic acid.

Acetic aldehyde is miscible in all proportions with water, alcohol, and ether. It is insoluble in a saturated solution of calcium chloride, but this property is not available for the quantitative separation of aldehyde from alcohol. A better method is to treat the liquid with dry calcium chloride, which forms a compound with the alcohol, when the aldehyde may be distilled off by the heat of a water-bath.

When kept in closed vessels, aldehyde often passes into liquid or solid polymers, especially in presence of traces of mineral acid. An alcoholic solution is tolerably permanent. Dehydrolyzing agents, such as phosphoric anhydride and concentrated sulphuric acid, when heated with

aldehyde turn it thick and black, but it may be distilled from sulphuric acid diluted with an equal weight of water.

Aldehyde is a powerful reducing agent. It separates metallic silver from the ammonionitrate, when gently warmed, an acetate being formed in the liquid. The reaction is rendered more delicate by the addition of alkali. A suitable mixture may be prepared by mixing equal measures of 10% aqueous solutions of silver nitrate and sodium hydroxide, and then adding ammonia drop by drop till the oxide of silver is dissolved. The reagent should be freshly prepared, as it is liable to decompose with deposition of fulminating silver. It yields an immediate mirror with a liquid containing 1% of aldehyde, and in half a minute with a solution containing 1 in 1000, while 1 part of aldehyde in 10,000 of water yields a yellow-brown mirror in five minutes. The solution to be tested should be previously distilled, as several varieties of sugar slowly reduce the reagent.

Aldehyde gives a copious precipitate of cuprous oxide when heated with Fehling's solution.

When in alcoholic or aqueous solution, aldehyde is conveniently detected by its reaction on heating with sodium hydroxide. When thus treated, the liquid becomes yellow and turbid, and a reddish-brown resinous mass rises to the surface, the liquid emitting a highly disagreeable odour. The solution contains formate and acetate. This formation of aldehyde-resin is the characteristic reaction of aldehyde, and has been utilised by J. C. Thresh (*Pharm. Jour.*, [3], 1878, 9, 409, 9 (1878-9), 409), for its estimation. To effect this, 1 part of pure aldehyde should be diluted with 200 measures of water, 30 measures of a syrupy solution of sodium hydroxide added, and the whole heated and kept at the b. p. for a few seconds. It is then allowed to cool, and after two hours is diluted with 200 measures of warm methylated spirit (free from aldehyde), and then made up to 500 measures by addition of water. This solution is quite clear, and of a reddish-yellow color. As it quickly alters, it is desirable to make a solution of potassium bichromate of the same tint, and employ that instead of the original liquid. To determine aldehyde, the liquid containing it, suitably diluted and previously distilled if necessary, is treated in exactly the same manner as the pure aldehyde, and the colour of the liquid obtained compared with the standard, and the darker diluted with water till the tints are identical. The comparison is effected in much the same manner as in Nessler's test.

Rocques' Method of Estimating Aldehyde (*J. Pharm. Chim.* [6], 1898, **8**, 390, 497).—An alcoholic sulphite solution is prepared by dissolving 12.6 grm. sodium sulphite in 400 c.c. of water, adding 100 c.c. of N/1 sulphuric acid, and making to 1000 c.c. with 95% alcohol of the highest purity. The solution is allowed to stand overnight and filtered from the sodium sulphate.

A suitable volume of the sample, which should not contain more than 2% of aldehyde, is placed in a flask marked at 100 c.c., an accurately measured volume of the alcoholic sulphite solution is added, and the liquid is made up to 100 c.c. with 50% alcohol of highest purity. The flask should have a long neck and be well closed after the mixture is made up. A similar flask is prepared with the diluted reagent alone. The flasks are kept at 50° for four hours, cooled, and the contents titrated with standard iodine with starch in the usual manner. Rocques uses different strengths of iodine according to the amount of aldehyde present, but for ordinary cases he prescribes N/1 solution. If the sulphite solution has been properly made, it and the iodine solution will be equivalent volume for volume. 1 c.c. of N/1 iodine equals 0.0032 of sulphur dioxide or 0.0022 of acetic aldehyde.

For a method of preparing a standard aldehyde solution and for further information in reference to estimation of aldehyde, see p. 198.

Aldehyde also combines with ammonia (NH_3) forming a crystalline substance of the formula C_2H_4O,NH_3, or $CH_3.CH(NH_2).OH$ (amido-ethyl alcohol), insoluble in ether and decomposed on distillation with moderately dilute sulphuric acid.

For a special process for separating formaldehyde and acetaldehyde, see page 264.

Betanaphthol test (Mülliken, *Ident. Pure Org. Comp.*, vol. 1), about 0.25 grm. betanaphthol, 2 drops hydrochloric acid and 20 c.c. glacial acetic acid are shaken until the naphthol is all dissolved. A drop of the solution to be tested is then added and the liquid heated to between 50° and 60° for 5 minutes, boiled for 1 minute, cooled and shaken vigourously until a precipitate settles. As the liquid tends to show the phenomenon of supersaturation, it will be well to allow considerable time and to stir actively with a glass rod before deciding that the result is negative. If a precipitate appears, it should be collected on a filter previously moistened with glacial acetic acid, washed with 1 c.c. of the same, then boiled with a mixture of 3 c.c. alcohol and 1 c.c. water for half a minute. Much of the precipitate may not dis-

solve. The solution is cooled thoroughly, shaken actively, and the precipitate that separates collected on a filter, washed with 1 c.c. cold dilute alcohol (1 to 1) and dried for 30 minutes at 100°. If acetic aldehyde was present in the liquid tested, the product is ethylidene-dibetanaphthol oxide $(C_{10}H_6)_2O(C_2H_4)$, which melts at 172.5° to 173.5°.

Paraldehyde. $C_6H_{12}O_3$.—This polymeride is produced by adding a minute quantity of hydrochloric or sulphurous acid to ordinary aldehyde. Also, on adding a drop of concentrated sulphuric acid to aldehyde violent ebullition occurs, much aldehyde is volatilised, and the residue consists of paraldehyde. Zinc chloride acts similarly, but calcium chloride and potassium acetate do not. The paraldehyde may be purified from unchanged aldehyde by cooling the liquid below 0°, when the crystals which separate are pressed between folds of blotting-paper and distilled.

Paraldehyde is a colourless, transparent liquid with strong odour and sharp taste. Its sp. gr. is 0.990 at 25° (United States Pharmacopœia). It is soluble in 8 parts of cold and 16.5 parts of boiling water; on account of the lower solubility at the higher temperature, the cold saturated solution becomes turbid on boiling. Paraldehyde is miscible in all proportions with alcohol and ether. It solidifies at about 0°, melts at 10.5° and boils at between 121° and 125°. The vapour is inflammable. The liquid is nominally neutral, but may be slightly acid to litmus. It possesses reducing power similar to ordinary aldehyde. The United States Pharmacopœia requires that 1 c.c. of paraldehyde shall form with 10 c.c. of water a clear solution, which must not precipitate with silver nitrate or barium chloride.

Metaldehyde, xC_2H_4O, is another polymeride produced simultaneously with paraldehyde (see above). It is insoluble in water, and almost insoluble in alcohol or ether, but dissolves somewhat in acetaldehyde. Its best solvents are hot chloroform and benzene. At ordinary temperatures the crystals are permanent in the air. It is reconverted more or less completely into ordinary aldehyde by repeated distillation or by heating in a sealed tube to 110° or 115° and readily by distillation with a little dilute sulphuric acid. Permanganates, chromic acid mixture, and ammonium hydroxide are without effect on metaldehyde, but chlorine at once converts it into ordinary chloral. With a hot strong solution of alkali, metaldehyde very slowly yields aldehyde-resin, the reaction being probably preceded by a formation of ordinary aldehyde.

Acetal, $C_6H_{14}O_2$, has the constitution of a di-oxyethyl-acetaldehyde: $CH_3.CH(OC_2H_5)_2$. It is produced by the action of aldehyde on alcohol, and hence is a constituent of crude spirit and of the "feints" obtained in the rectification of alcohol. When pure, acetal is a liquid of pleasant taste and odour, boiling at about 105° and having a density of 0.821 at 22°. By oxidising agents it is converted into acetic acid and aldehyde, and when heated with acetic acid, it yields ethyl acetate and aldehyde. If a dilute aqueous solution be treated with sodium hydroxide and iodine a clear colourless liquid is formed, which yields a dense precipitate of iodoform when acidified. From alcohol, acetal may be separated by distillation over dry calcium chloride and from aldehyde and ethyl acetate by heating the liquid with strong solution of potassium hydroxide.

Dimethyl-acetal occurs in crude wood spirit in proportions ranging from 1 to 2%.

CHLORAL.

Trichloraldehyde. C_2HCl_3O.

Chloral is obtained in practice by the prolonged action of dry chlorine on absolute alcohol. When the liquid acquires a sp. gr. of 1.400 it is distilled with an equal weight of strong sulphuric acid, the fractions passing over below 94° being kept separate, and the process stopped when the temperature rises to 100°. The distillate is neutralised with calcium carbonate and again distilled. The reactions which occur in the manufacture of chloral are very complicated, and secondary products are liable to be formed.

Chloral is a colourless oily liquid, sp. gr. 1.544 at 0°, or 1.502 at 18°. It boils at 94.4° and distils unaltered. It is soluble in ether or chloroform without change.

When kept for some time, or when left in contact with moderately concentrated sulphuric acid, chloral is converted into an insoluble polymeric modification called metachloral, which is insoluble in cold and but sparingly soluble in boiling water and insoluble in alcohol or ether even when boiling. Pure chloral does not become polymerised, and the change is also said to be prevented by addition of a little chloroform. When heated to 180° metachloral distils with reversion to liquid chloral. By the action of alkalies chloral yields chloroform and a formate.

If an aqueous solution of chloral is heated to 50° with zinc, and very dilute acid gradually added, aldehyde and paraldehyde are formed and may be distilled off.

When chloral is mixed with an equivalent quantity of absolute alcohol it is converted into—

Chloral Alcoholate.

This substance forms white crystals, which melt at 46°. It boils at 113.5°. These properties serve among others, to distinguish it from—

Chloral Hydrate. Trichlorethylidene glycol.

This substance results from the mixture of equivalent quantities of chloral and water. The mixture becomes heated and solidifies to a mass of crystals. The term "chloral hydrate" is a misnomer; the substance is not a combination of water and chloral, but a chlorinated diatomic alcohol. The term is, however, too firmly fixed in medical and pharmaceutic literature to be avoided, and hence it will be used in this work.

Chloral hydrate is soluble in 1.5 times its weight of water and is also soluble in alcohol, ether, benzene, petroleum spirit, and carbon disulphide. When crystallised from the last solution it boils at 97.5°. When mixed with an equal weight of camphor or phenol it rapidly liquefies. The liquid has the mixed odour of its constituents and does not precipitate silver nitrate.

Chloral hydrate is soluble with difficulty in cold chloroform, requiring four times its weight; a fact which distinguishes it from the alcoholate, which is readily soluble in chloroform. The alcoholate represents less chloral than the hydrate.

Chloral hydrate and alcoholate should be completely volatile and their aqueous solutions should be perfectly neutral to litmus.

Aqueous solution of chloral hydrate gives no reaction with silver nitrate in the cold, but on boiling and adding a little of ammonium hydroxide a mirror is readily produced. If kept some time, chloral hydrate contains a trace of hydrochloric acid, and the solution in water then gives a cloud with silver nitrate, but the production of a distinct precipitate indicates serious impurity.

When water is present chloral hydrate is deliquescent, and in warm weather even melts. Hence it is often made with a slight deficiency of water. If more than a shade short of this the product has a tendency to become acid, and ultimately partially insoluble from formation of metachloral.

In the following table are given other useful distinctions between chloral alcoholate and chloral hydrate:

	Chloral alcoholate	Chloral hy-lrate
1. M. P.	46°	48°-49°
2. B. P.	113.5°	97.5°
3. Sp. gr. of the fused substance at 66°:	1.344	1.57
4. Sp. gr. of the aqueous solution at 15.5°:		
5 %	1.007	1.019
10 "	1.028	1.040
15 "	1.050	1.062
20 "	1.071	1.085
5. Gently heated with nitric acid of 1.2 sp. gr.	Violently attacked.	Scarcely acted on.
6. Shaken with an equal volume of strong sulphuric acid.	Brown.	No visible change.
7. Warmed with two volumes of water.	Melts without complete solution, and on cooling congeals below the surface.	Readily dissolved.
8. Heated on platinum foil.	Inflames readily.	Scarcely burns.
9. With alkali and iodine.	Gives iodoform.	Gives no iodoform.

The solidifying point of melted chloral hydrate is an indication of some value. The sample should be placed in a small test-tube, fused, and the tube immersed in water at about 55°. A thermometer is placed in the liquid, and the temperature at which it becomes opalescent noted. The best quality solidifies at about 48° to 49°, and the best *practically* adjusted specimens within half a degree of 50°. A low freezing point indicates excess of water, and such specimens are liable to deliquesce. Small granular crystals and saccharoid masses are purer than large crystals or needles.

The b. p. is also of service as a test of purity. The sample should be placed in a test-tube with some broken glass. A pure sample will begin to boil rapidly, 97°, and the temperature will change but little until one-half has been volatilised. The material, however, undergoes slow decomposition at the b. p., so that the first portions of the distillate are underhydrated, and the last overhydrated. The b. p. consequently undergoes a gradual rise. The best commercial specimens, *i. e.*, those slightly underhydrated, begin to boil throughout the liquid at about 965°. The underhydrated portion boils off in a few

seconds, and the b. p. rises to 97°, and finally to 97.5° or 98°, by the time half has boiled away. A b. p. above 98° indicates an over-hydrated and deliquescent sample. If the boiling fairly commences below 95°, the sample is too much underhydrated, and is liable to decompose on keeping.

Detection and Estimation of Chloral.—The detection and estimation of chloral have acquired considerable importance of recent years on account of the not infrequent employment of the substance for drugging liquor to facilitate the commission of robbery or rape.

Chloral hydrate may be detected by the same means as chloroform (page 274). It reduces Fehling's solution on heating. The reaction may be employed to detect traces of chloral if other reducing substances are absent, and might probably be made quantitative.

Traces of chloral may be detected by Hofmann's test for chloroform (see page 274); also, by boiling the liquid and passing the vapour through a red-hot tube, when hydrochloric acid will be formed, and the condensed water will precipitate silver nitrate.

For the estimation of real *chloral* yielded by the hydrate, advan-may be taken of the reaction with alkalies, which results in the separation of chloroform and the production of a formate.

K. Müller gives the following method: 25 grm. of the sample are placed in a finely-graduated tube, and a strong solution of potassium hydrate added, in quantity rather more than sufficient for the above reaction. A large excess must be avoided. The tube must be kept well cooled, as the action is very violent at first. Afterwards, the tube may be closed and the mixture shaken. After resting an hour or two the liquid becomes clear and separates into two layers. The lower layer is chloroform, and, after being brought to a temperature of 17°, the volume may be read off. Its sp. gr. is 1.491, and hence the measure of chloroform in c.c., multiplied by 1.84, gives the grms. of anhydrous chloral in the quantity of the sample employed. If the factor 2.064 be substituted, the product will be the weight of chloral hydrate present. Müller obtained by this process an average of 71.6 % of chloroform from pure chloral hydrate, against 72.2 % as required by theory.

C. H. Wood proposed the following: 10 grm. of the sample are dissolved in 50 c.c. of water contained in a small flask, and 4 grm. of slaked lime is added. A cork with a tube bent twice at right angles is

adapted to the flask, the outer end of the tube being somewhat drawn out and immersed in a small quantity of water, contained in a narrow graduated glass tube surrounded with cold water. A gentle heat is applied to the flask, and the chloroform slowly distilled over. After a few minutes the heat is gradually increased, so as to keep the mixture boiling, the operation being continued till 10 c.c. measure has passed over. Nothing remains but to bring the chloroform to the proper temperature and read off the volume. The addition of a few drops of potassium-hydroxide solution destroys the meniscus of the chloroform, and enables the operator to observe the measure accurately. The process is brief. Too much lime occasions frothing, but an excess appears to have no decomposing action on the chloroform. Lieben's iodoform test for alcoholate is readily applied to the aqueous portion of the distillate. Allen found this plan convenient and fairly accurate. A correction may advantageously be made for the slight solubility of chloroform. This is about 0.3 c.c. for every 100 c.c. of aqueous liquid.

A simple and satisfactory modification of the process has been suggested by M. Meyer, and has given satisfactory results. It has the advantage of being applicable to small amounts of material. One or 2 grms. of the sample are dissolved in water, and free acid removed by shaking the liquid with barium carbonate and filtering. The filtrate is treated with a moderate excess of normal sodium hydroxide, and titrated back with acid in the usual way, litmus being used as an indicator. Each c.c. of normal alkali neutralised by the sample corresponds to 0.1475 grm. of chloral (C_2HCl_3O), or 0.1655 grm. of chloral hydrate.

Other processes of assaying chloral hydrate have been based on its decomposition by ammonium hydroxide and on its conversion into chloral by sulphuric acid, but they are liable to error, and are not better than the methods described.

Trichloracetic acid.—This is a product of the action of oxidising agents on chloral. When equivalent quantities of chloral hydrate and potassium permanganate are cautiously mixed in concentrated solution, potassium trichloracetate is formed, and may be obtained in white silky crystals by filtering and evaporating the liquid. By the action of alkalies, trichloracetic acid yields chloroform and a carbonate, and responds to all other tests for chloral dependent on its conversion into chloroform.

The acid may be obtained by decomposing the potassium salts in the

usual way. It forms colourless crystals, very deliquescent. The solution is powerfully acid and corrosive. It coagulates albumin and is one of the most delicate tests for this substance. Assay of the acid may be made by titrating standard alkali and applying the methods used in assaying chloral hydrate.

Butyric Chloral. Butyl Chloral.—Butyric trichloraldehyde. This is erroneously called croton chloral. When chlorine is passed into aldehyde, this substance is formed in addition to ordinary chloral. It bears the same relation to butyl alcohol and butyric acid that ordinary chloral bears to ethyl alcohol and acetic acid.

Butyl chloral was at first called croton chloral, the hydrogen being underestimated, which led to the supposition that it was the trichlorinated aldehyde of crotonic acid, the fourth member of the acrylic or oleic acid series.

Butyric chloral is a dense, oily liquid of peculiar odour, boiling at about 163°. When treated with a considerable excess of warm water it dissolves, and on cooling deposits

Butyric Chloral Hydrate.

This substance forms white, silvery crystalline scales melting at 78° and having a sweetish melon flavour. The sp. gr. is 1.695, that of solid chloral hydrate being 1.818. Butyric chloral hydrate is but little soluble in cold water, but more so in hot. Its solubility is increased by addition of glycerol. It is very soluble in alcohol and ether, but insoluble, or nearly so, in chloroform. This last property may be employed to separate it approximately from ordinary chloral hydrate. It differs also from the latter body in its m. p. and b. p. The two bodies may also be separated by distillation, ordinary chloral hydrate passing over a little below 100°, while butyric chloral hydrate is decomposed into water, which distils at about 100°, and anhydrous butyric chloral boiling at about 163°.

When acted on by alkalies, butyric chloral hydrate is at first decomposed with production of a formate and propylic chloroform, but this again splits up with formation of a metallic chloride and allylene dichloride.

Allylene dichloride is very unstable, being gradually decomposed even at ordinary temperatures, and acquiring an acid reaction and disagreeable odour. The proneness to change, so marked in some samples of commercial chloroform, and the readiness with which the latter decomposes and becomes acid, are properties possibly due to

the presence of allylene dichloride. Its presence may be due to the existence of aldehyde in the crude alcohol used for the preparation of the chloroform. By the action of chlorine the aldehyde is converted into butyl chloral, and this, by subsequent contact with the calcium carbonate used for neutralisation, gives allylene dichloride.

Chloralformamide.

This is produced by the action of formamide (CH_3NO) on chloral. It forms colourless, odourless crystals, soluble in about 19 parts of water at 25°. The solution is somewhat bitter and is neutral to litmus. One part of chloral-formamide dissolves in 1.3 parts of alcohol, and it is also readily soluble in ether, glycerol, ethyl acetate, and acetone. Heated alone, it melts at about 115° and at a higher temperature volatilises leaving no appreciable residue. Its solution in water is not affected by acids, but is decomposed by sodium hydroxide with formation of chloroform. The solution in alcohol does not redden litmus nor produce a precipitate with solution of silver nitrate.

CHLOROFORM.

Trichlormethane. Methylene terchloride.

Chloroform was formerly made by distilling dilute alcohol with calcium hypochlorite and calcium hydroxide, but it is now prepared largely from acetone and from chloral.

Chloroform is a colourless liquid of marked odour and sharp, sweetish taste. It is very volatile. The vapour is not combustible alone, but in mixture with alcohol vapour, burns with a smoky flame, edged with green. According to Landolt and Börnstein's *Tabellen*, chloroform has a sp. gr. 1.5264 at 0°/4°, melts at 70° and boils at 61.2° (corrected).

Chloroform is soluble in about 200 volumes of cold water (0.44 grm. in 100 c.c.), to which it imparts a sweet taste. It is miscible in all proportions with absolute alcohol, ether, benzene, and petroleum spirit. It is soluble to a limited extent in dilute alcohol. It dissolves many organic bases, fats, waxes, resins, camphor, india-rubber, gutta-percha, iodine, bromine, and phosphorus.

Detection and Estimation of Chloroform.—As a rule, the detection of chloroform itself is less important than the recognition and estimation of other substances in presence of chloroform.

A very delicate method for the detection of chloroform in presence of large quantities of alcohol has been described by A. W. Hofmann.

All that is necessary is to add some alcoholic sodium hydroxide and a little aniline to the liquid to be tested. Either immediately or on gently warming the mixture, a strong and peculiar smell will be observed, due to the formation of phenyl carbamide (phenyl isocyanide), Bromoform and iodoform give the same reaction, as also do chloral, trichloracetic acid, and all other bodies which yield either of the above products by treatment with alkalies, but ethylidene chloride, $C_2H_4Cl_2$, gives no isonitrile under these conditions. The test is so delicate that one part of chloroform dissolved in 5,000 parts of alcohol may be detected with certainty by means of it.

Reduction of Fehling's solution is also a test for chloroform. When the solution is heated, the cuprous oxide separates promptly. Chlorethylidene and alcohol do not interfere with the test.

When chloroform vapour mixed with hydrogen is passed through a red-hot tube, is it decomposed with production of hydrochloric acid. This reaction is used for the detection and estimation of chloroform. The sample should be boiled in a small flask through which a current of hydrogen is allowed to pass. The mixed hydrogen and chloroform vapour are then caused to traverse a short length of heated combustion tube containing platinum wire-gauze or loose asbestos.

The products are passed through a bulb-tube containing water, and the hydrochloric acid is titrated with standard alkali, or precipiated with silver nitrate. 109.5 parts of hydrochloric acid, or 430.5 of silver chloride represent 119.5 of chloroform. Berthelot points out that the reaction with silver is apt to be vitiated by the presence of acetylene and hydrocyanic acid, and recommends that the aqueous solution of the gases should be well boiled before adding silver nitrate.

This process is especially useful for the estimation of small quantities of chloroform contained in other non-chlorinated liquids. It may be employed for the detection and estimation of chloroform in blood. When its detection only is required, a current of air may be substituted for the hydrogen. There is no occasion to heat the blood.

Vitali suggests that the mixture of hydrogen with chloroform vapour obtained as in the last reaction should be submitted to Hofmann's isonitrile reaction or passed through a freshly prepared mixture of thymol and solid potassium hydroxide, when if chloroform is present the mixture will be coloured a fine reddish-violet.

When chloroform is added to a solution of a- or β-naphthol in strong

potassium hydroxide, and the liquid is heated to about 50°, a fine blue is developed, changing in contact with the air to blue-green, green, green-brown, and finally brown.

Commercial Chloroform.—The United States Pharmacopœia preparation consists of "99 to 99.4 %, by weight, of absolute chloroform and 0.6 to 1 % of alcohol."

Many specimens of commercial chloroform undergo more or less change on keeping. According to Personne, samples liable to alteration contain chloro-carbonic ether, $C_2H_5CO_2Cl$. The change has also been attributed to the presence of allylene dichloride. Specimens of chloroform, originally of good quality, become on keeping impregnated with hydrochloric, hypochlorous, and formic acids. J. Regnauld has found that carbon oxychloride, $COCl_2$, was readily produced by the action of ozonised air on chloroform, and considers the accidental presence of this body in chloroform very common. He has also found that very carefully prepared chloroform can be kept unchanged if exposed to air or light simply, but that the combined action of air and light rapidly affects the purity of the preparation. The change is entirely prevented by the addition of a little alcohol.

In addition to the impurities resultant from decomposition by keeping, commercial chloroform may contain alcohol, aldehyde, and various chlorinated bodies. These last are very injurious and even poisonous, and are detected and eliminated with considerable difficulty. Other products may be present if the alcohol employed for the manufacture of the chloroform contained methyl or amyl compounds. Alcohol and aldehyde are sometimes added to chloroform in very considerable proportions. The adulteration of chloroform with ether and acetic ether has also been practised.

Free chlorine and hypochlorous and hydrochloric acids in chloroform may be recognised by shaking the sample with a solution of silver nitrate which in presence of either of the above impurities will produce a white precipitate, whereas chloroform itself gives no reaction with silver nitrate, either in aqueous or alcoholic solution. If the precipitate blacken on heating the presence of *aldehyde* or *formic acid* is indicated. Free chlorine and hypochlorous acid are distinguished from hydrochloric acid by their power of bleaching instead of merely reddening litmus, and by liberating iodine from a solution of pure potassium iodide when the sample is shaken with it. The liberated iodine colours the chloroform reddish-violet.

Ethylene dichloride, $C_2H_4Cl_2$, may be detected by drying the sample by agitation with dry potassium carbonate and then adding potassium. This does not act on pure chloroform, but ethylene dichloride produces chlorethylene, C_2H_3Cl, a gas of an alliaceous odour. It is doubtful if the substance in chloroform of the formula $C_2H_4Cl_2$ is always ethylene dichloride. It may be the isomer, ethylidene chloride, $CH_3.CHCl_2$.

The presence of *ethyl chloride*, in chloroform is best recognised by distilling the sample with water in a water-bath. The first portions of the distillate will have a distinct smell of the foreign body.

"Methylated chloroform" is chloroform prepared from wood spirit or methylated spirit. It is a mistake to suppose that methylated chloroform has received an actual addition of wood spirit, but such chloroform is liable to be much less pure than that obtained solely from ethyl alcohol.

Imperfectly purified methylated chloroform is specifically lighter than the pure substance, has an empyreumatic odour, and produces disagreeable sensations when inhaled. In some cases such chloroform seems actually poisonous and produces general and rapid prostration. Such impure chloroform contains a notable amount of a chlorinated body, lighter than water and boiling at a much higher temperature than chloroform. A similar but different oil (heavier than water) is sometimes contained in much smaller quantity in chloroform prepared from alcohol containing no methyl compounds.

Owing to the poisonous actions of several methyl derivatives, it is inadvisable to use for medicinal purposes chloroform made from methylated spirit.

Chloroform is not soluble in strong sulphuric acid and, when pure, is not acted on until after the lapse of some time when shaken with that reagent. Any darkening of the acid which occurs may be due to the presence of *aldehyde, wood spirit,* or chlorinated oils. Pure chloroform floats on strong sulphuric acid with a contact-surface convex downwards, but if impure gives a plane contact-surface. The United States Pharmacopœia adds the following to this test:

2 c.c. of the sulphuric acid separated from the chloroform, diluted with 5 c.c. of distilled water, should remain colourless and clear, and while hot from the mixing, should be odourless or give but a faint vinous or ethereal odour. When further diluted with 10 c.c. of distilled water, the liquid should remain clear and should not be affected by silver-nitrate solution.

The b. p. of chloroform is a valuable indication of its purity.   Chloroform boils at 60.8°.   The presence of $1/2$% of alcohol reduces the b. p. to 59.8° or 60°.   A b. p. higher than 61° indicates the presence of *amyl* or *butyl compounds*.   In some cases the b. p. of the last portions distilled is as high as 70°.

Chloroform volatilises entirely without disagreeable odour.   The impurities are generally less volatile.   Many common impurities in chloroform may be recognised by the odour left on the evaporation of the sample from filter-paper soaked with it.

Chloroform is not *visibly* altered when heated with solution of potassium hydroxide, but it is slowly acted on with formation of formate and chloride.   In alcoholic solution this reaction occurs rapidly.

Any considerable admixture of *ether* with chloroform would be indicated by the inflammability and diminished density of the liquid.

Chloroform does not change the colour of an alkaline solution of potassium permanganate from violet to green within half a minute, but as the change is caused by alcohol equally with more objectionable impurities, the reaction has little practical value.

The most delicate test for the presence of *alcohol* in chloroform is that of A. Lieben as modified by Hager.   The sample should be agitated with five measures of water, the liquid passed through a wet filter, and the filtrate examined as described on page 105.

Potassium hydroxide is quite insoluble in dry chloroform, but dissolves sensibly in presence of *water* or *alcohol*.   A little of the solid is fused on a loop of platinum wire and introduced into chloroform contained in a dry test-tube, the liquid will not acquire the power of turning red litmus-paper blue unless water or alcohol be present.   If more than a trace of alcohol is present, the decanted chloroform, when shaken with water, yields a liquid which gives a blue precipitate with a solution of copper sulphate.   To use this test with certainty to distinguish between water and alcohol the sample must be first shaken with recently ignited potassium carbonate.   This treatment will remove water but not alcohol, so that if the chloroform still possesses the power of dissolving the alkali alcohol is present.

Oudemanns proposed to ascertain the alcohol in commercial chloroform by shaking 10 c.c. of the sample in a flask with an excess of pure dry cinchonine.   The flask is kept for an hour at a temperature of 17°, with frequent agitation.   The liquid is then passed through a dry filter, and 5 c.c. of the filtrate evaporated to dryness in a small hand beaker.

The following are the amounts yielded by 5 c.c. of chloroform containing different proportions of alcohol:

Residue	Alcohol	Residue	Alcohol
Mg.	%	Mg.	%
21	0	260	6
67	1	290	7
111	2	318	8
152	3	343	9
190	4	346	10
226	5		

Stoedeler has suggested fuchsine for detecting alcohol in chloroform. The sample becomes coloured red if alcohol is present, the depth of colour varying with the proportion of alcohol. Allen found (*Analyst*, (1877, 22, 97) that, even after agitation with calcium chloride, chloroform coloured on adding fuchsine, but by agitating a sample with 1/5 of its bulk of strong sulphuric acid, and subsequently removing traces of the latter by shaking with dry precipitated barium carbonate, a liquid was obtained so pure as to give only a very slight colouration. This purified chloroform can be used in a similar manner to ether for estimating small proportions of alcohol in chloroform. Chloroform may also be purified from water, alcohol, and ether by agitating with sulphuric acid as above, separating the acid, shaking the chloroform with a strong solution of sodium carbonate, and, lastly, distilling it over freshly burnt lime.

Chloroform can be freed from water and alcohol by the same processes recommended for purifying ether (page 227). The reaction with calcium carbide (page 110) may also be of use.

When the quantity of alcohol in chloroform exceeds 1 or 2%, the proportion may be ascertained with tolerable accuracy by shaking 20 c.c. of the sample in a graduated tube with 80 c.c. of water. If the chloroform is pure it will collect at the bottom in clear globules, but in the presence of alcohol the liquid and the surface of the drops will become dim and opalescent. The reduction in the volume of the chloroform shows the proportion of alcohol in the amount taken. The addition of a few drops of potassium hydroxide solution destroys the meniscus and enables the volume to be read more accurately. The aqueous liquid may be tested for sulphuric acid by barium chloride

for free chlorine or hypochlorous acid by starch and potassium iodide; for hydrochloric acid by silver nitrate; and the presence of alcohol definitely proved by the iodoform test.

The proportion of *alcohol* present in chloroform can in some cases be ascertained from the sp. gr. According to C. Remys, the presence of 1/8 % of alcohol reduces the sp. gr. by .002, and 1/2 % by .008. According to A. H. Mason, chloroform containing 1 % of alcohol has a sp. gr. of 1.497 at 15.5° C. The chloroform of the British Pharmacopœia has a sp. gr. of 1.49. Chloroform containing amyl or butyl compounds has a higher sp. gr. than 1.500.

Chloroform has marked antiseptic powers and is especially convenient for preserving urine samples. A few drops well shaken with 100 c.c. will be sufficient to preserve the liquid for an indefinite time. An excess should be avoided, as the globules collect at the bottom of the bottle and interfere with the examination of the sediment. It does not simulate the common tests except those with copper solutions, nor interfere with any but fermentation. The bismuth and phenylhydrazine tests give no result with a solution of chloroform in urine free from sugar. Saccharine urine in an active state of fermentation is brought to quiescence by addition of chloroform. The liquid may be freed from the preservative by adding water and boiling down to the original volume.

Spirit of chloroform, British Pharmacopœia, is a solution of chloroform in 19 measures of rectified spirit (55° O. P.) and should have a density of 0.871. A lower sp. gr. may be due to deficiency of chloroform or to the use of spirit of 60° O. P. "Chloric ether" is a spirituous solution of chloroform of uncertain strength.

Spirit of chloroform, United States Pharmacopœia, consists of 60 c.c. chloroform and 940 c.c. of alcohol.

The proportion of chloroform present in spirit of chloroform, "chloric ether," and similar preparations may be ascertained with accuracy by introducing into a narrow graduated tube 20 c.c. of the sample and 30 c.c. of dilute sulphuric acid (1 to 6) coloured with a little fuchsine. A cork is then inserted and the contents of the tube thoroughly shaken. When the chloroform has separated, the tube is tapped to cause any floating globules to sink, and about 10 c.c. of petroleum spirit is cautiously poured on the surface of the acid. The cork is reinserted and the volume of petroleum spirit employed is carefully noted, when the contents of the tube are well mixed by agitation. After separation the

volume of petroleum spirit is again observed, when its increase will be due to the dissolved chloroform. Better results are obtainable in this way than without petroleum spirit, but great care is necessary to avoid error from expansion or contraction through alteration of temperature. Hence, before observing the volume of petroleum spirit originally used, and again before the final reading, the tube should be immersed in a cylinder of cold water for a short time. The process gives inaccurate results when the proportion of chloroform exceeds about 30%. In such cases the method given on page 275 should be employed.

The chloroform in mixtures of chloroform and alcohol may also be determined by decomposition with alkali in the manner described on page 278.

Methylene Dichloride. Methene Dichloride. Dichlormethane. CH_2Cl_2.

Methylene dichloride is obtained by exposing the vapour of methyl chloride in admixture with chlorine to the action of daylight in a large glass globe. The products are passed through two Woulffe's bottles, and then into a flask surrounded by a freezing mixture. The former chiefly retain chloroform, while the methylene dichloride condenses in the flask. It may also be obtained by the reduction of chloroform in alcoholic solution by zinc and hydrochloric acid.

Methylene dichloride is a powerful anæsthetic. Being more expensive than chloroform, the latter liquid is sometimes substituted and sold for the former, which it closely resembles in odour. The two bodies may be distinguished by their sp. gr. and b. p. The methylene dichloride burns with a smoky flame and dissolves iodine with brown colour, while chloroform unmixed with alcohol burns with great difficulty, giving a green-edged flame, and dissolves iodine forming reddish-violet liquid.

A mixture of alcohol and chloroform has been substituted for methylene dichloride. On shaking the sample with water, the alcohol would be dissolved, and the chloroform would then be recognisable by its sp. gr.

Bromoform. Tribromomethane.

This body closely resembles chloroform, but boils at 150° to 152° Its sp. gr. is 2.9 at 12° or, according to E. Schmidt, 2.775 at 14.5°. It solidifies at +9°.

Alkalies convert bromoform into chloride and formate. By the

action of alcoholic potassium hydroxide, gas is evolved, consisting of one volume of carbon monoxide and three of ethylene.

Bromoform has been found in commercial bromine, (Hermann, *Annalen*, 1855). It may be detected by fractional distillation of the bromine on the water-bath or by treating the sample with excess of solution of potassium iodide, and then adding sufficient sodium thiosulphate to take up the iodine set free. The characteristic odour of bromoform then becomes apparent. The impurity now rarely, if ever, occurs.

Iodoform.   Triiodomethane.

Iodoform is produced in Lieben's test for alcohol (page 105). It may be conveniently prepared by heating a mixture of 1 part of iodine, 1 of alcohol, 2 of crystallised sodium carbonate, and 10 of water to about 70 to 80°, until decolourised, when the iodoform separates as lemon-yellow powder, which may be filtered from the liquid, washed with cold water and dried.

Iodoform is a light yellow, shining, crystalline solid, having a persistent unpleasant odour. It sublimes at a gentle heat without change, distils with vapour of water, and volatilises sensibly at ordinary temperatures. Heated strongly, it is decomposed with formation of violet vapours of iodine, and deposition of carbon. It is nearly insoluble in water (about 1 part in 10,000) and dilute alkaline and acid liquids; sparingly soluble in alcohol (1 in about 50), but more readily in absolute alcohol (1 in 25); and with facility in ether, chloroform, and carbon disulphide. It is also dissolved by many essential oils, and sparingly by glycerol, benzene, and petroleum spirit.

Iodoform is employed in medicine especially as an antiseptic dressing. Its chemical reactions closely resemble those of chloroform. Its microscopic appearance is very characteristic, the usual forms being hexagonal plates, stars, and rosettes.

Iodoform may be extracted from urine and other aqueous liquids by agitation with ether. On allowing the ethereal layer to evaporate spontaneously, the iodoform may sometimes be recognised by examining the residue under the miscroscpe. If no distinct forms are observable, the residue should be taken up with a little absolute alcohol, and three or four drops of the clear solution added to a minute quantity of a solution of phenol in sodium hydroxide. The mixture is cautiously heated, when a red deposit will be formed at the bottom of the tube, soluble in dilute alcohol with crimson colour.

Commercial Iodoform.—On agitation with water, iodoform should not yield a liquid precipitable, after filtration, by barium chloride or silver nitrate. It should leave no soluble residue on ignition in the air; and should be wholly soluble in boiling alcohol, but insoluble in brine.

Picric acid has been used as an adulterant of iodoform (*Pharm. Jour.* [3], 1883, **14,** 493). It may be detected by agitating the sample with dilute solution of sodium carbonate, carefully neutralising the filtrate with acetic acid, and adding potassium nitrate, when a yellow low precipitate of the sparingly soluble potassium picrate will be thrown down. The iodoform may also be separated by treating the sample with sodium hydroxide solution and agitating the liquid with chloroform, when only the picric acid will remain in the aqueous liquid. Picric acid may also be detected by the reddish-brown colouration produced on heating the cold aqueous solution of the sample with potassium cyanide.

SUGARS.

E. FRANKLAND ARMSTRONG, D. Sc., Ph. D., A. C. G. I.

Under the generic name of sugars is included a large number of substances occurring naturally in the animal or vegetable kingdoms or produced from the so-called *glucosides* by the action of ferments or dilute acids.

The sugars constitute a group of closely-allied compounds, in many cases distinguishable from each other only with considerable difficulty, while their quantitative separation is frequently impossible in the present condition of chemistry.

As a class, the sugars are crystallisable, readily soluble in water, somewhat less soluble or wholly insoluble in alcohol, and insoluble in ether and other solvents immiscible with water.

A *sweet taste* is possessed by nearly all sugars to a greater or less extent. Glycerol and glycol have a sweet taste, and, like the sugars, are polyatomic alcohols.

In many cases the sugars exert a powerful rotatory action on a ray of polarised light, the direction and extent of the rotation, being peculiar to each sugar. Hence the *optical activity* is a valuable means of estimating and differentiating sugars.

Constitution and Classification of Sugars.[1]—The sugars proper are aldehydes or ketones of hexatomic alcohols and may be obtained from these by limited oxidation or, conversely, converted into them by reduction. They are divided into three great groups, viz., (1) the mono-saccharides or glucoses; (2) the di-saccharides or saccharoses, and (3) the poly-saccharides; *e. g.*, starch, cellulose, etc. They mostly contain 6, or a multiple of 6, atoms of carbon, and hydrogen and oxygen in the proportion of 2 : 1.

[1] *Nomenclature.*—To avoid confusion in the following pages, the term sucrose has been used as the more scientific term for both cane and beet sugar, whilst the term cane sugar describes the commercial article irrespective of origin. The word glucose is restricted to the commercial product from starch, and dextrose is used for the pure sugar $C_6H_{12}O_6$, lævulose for the corresponding ketose derived from cane sugar. The disaccharide, milk sugar is also spoken of as lactose. Maltose is applied to the disaccharide derived from starch.

The abbreviation A. O. A. C. indicates the official and provisional methods of analysis adopted by the Association of Official Agricultural Chemists and published by the United States Department of Agriculture, Bureau of Chemistry as Bulletin 107, September, 1907, superseding Bulletins 46 and 65.

The Monosaccharides.—To this group belong the naturally occurring sugars containing 5 and 6 carbon atoms, known as pentoses and hexoses and also the closely related synthetical sugars with 3, 4, 7, 8, and 9 carbon atoms. They are characterised by the following general properties:

1. They are easily oxidised and reduce Fehling's solution.

2. They form with phenylhydrazine and acetic acid sparingly soluble crystalline osazones.

3. Those hexoses which occur naturally undergo alcoholic fermentation with yeast.

4. They form additive compounds with hydrogen cyanide.

The Disaccharides.—This group consists of sugars of the formula $C_{12}H_{22}O_{11}$; e.g., cane sugar, milk sugar, maltose, melibiose and others formed by the union of two monosaccharide residues through an oxygen atom. It may also be extended to include sugars, such as raffinose, formed by the union of three or more monosaccharide residues. The general properties of the members of this group are:

1. They are converted on hydrolysis by mineral acids or by specific enzymes into monosaccharides.

2. They are not directly fermentable unless first hydrolysed.

The Polysaccharides.—This group includes substances of high molecular weight, of the general formula $nC_6H_{10}O_5$ such as cellulose, starch, glycogen and dextrin. They are amorphous substances and yield simpler saccharides or ultimately monosaccharides on hydrolysis.

The following tables show the origin and leading characteristics of the more important mono- and disaccharides.

Isolation of Sugars.—The quantitative analysis of complex artificial or natural carbohydrate mixtures is one of the most difficult problems in organic analytical chemistry. Indirect methods have almost invariably to be employed and errors in the estimation are apt to become additive.

The general methods by which sugars are isolated in the proximate analysis of animal and vegetable substances depend much on the nature of the associated bodies. Principles of separation commonly utilised are: the removal of proteins bodies by heat or precipitation; the precipitation of dextrin and other gummy matters by alcohol; the removal of organic acids and various other matters by lead acetate; concentration of the solution with a view to promoting crystallisation; and the detection and estimation of the sugars present by their re-

MONO-SACCHARIDES (GLUCOSES).

Name	Origin and mode of formation	Specific rotation	Other characters
Aldohexoses, $C_6H_{12}O_6$. d-Dextrose.	Honey, sweet fruits. By action of acids on starch, cellulose etc.	$+52.7°$	Very soluble, slightly sweet, reducing power marked, easily fermentable. Forms sorbitol on reduction, gluconic acid on oxidation with bromine. Turns brown with alkalies. Forms a soluble phenylhydrazone and an insoluble osazone. Forms alkyl glucosides hydrolysed by enzymes.
d-Mannose	Ivory nuts.	$+14°$	Similar to dextrose. Forms an insoluble phenylhydrazone and the same osazone as dextrose. Forms alkyl glucosides not affected by enzymes.
d-Galactose	Action of acids on milk-sugar, gums.	$+81°$	Less soluble. Fermented with difficulty and not by all yeasts. Yields dulcitol on reduction and mucic acid on oxidation with nitric acid.
Ketohexoses, $C_6H_{12}O_6$. d-Lævulose.	Honey, fruits, inulin.	$-93.8°$	More soluble than dextrose. Crystallises with difficulty. Easily fermented. Properties similar to dextrose. Forms a methyl phenyl-osazone.
d-Sorbose.	Ripe mountain ash berries.	$-42°$	Not fermentable by yeast. Yields sorbitol on reduction.
Aldopentoses, $C_5H_{10}O_5$. l-Arabinose	Action of acids on gums.	$+105°$	Not fermented. Shows mutarotation. Forms arabonic acid on oxidation, arabitol on reduction. Yields furfuraldehyde on heating with hydrochloric acid.
l-Xylose.	Action of acids on straw.	$+19°$	Similar to arabinose. Forms xylonic acid on oxidation and xylitol on reduction.

DI-SACCHARIDES (Glucoses).—Continued.

Name	Origin and mode of formation	Specific rotation	Other characters
Non-reducing, $C_{12}H_{22}O_{11}$. Sucrose.	Sugar cane, beet, maple, etc.	+66.5°	Very soluble in water. Forms oxalic and saccharic acids on oxidation. Chars with conc. H_2SO_4. Very easily hydrolysed by dilute acids and by invertase to a mixture of dextrose and lævulose. Fermented only after inversion by yeast.
Trehalose.	Trehala manna, Fungi; *e. g.*, Aspergillus niger.	+197°	Hydrolysed by trehalase to two molecules of dextrose. It does not exhibit mutarotation or form an osazone.
Reducing, $C_{12}H_{22}O_{11}$. Maltose.	Starch, by the action of diastase or dilute acids.	+138°	Less soluble than dextrose. Exhibits mutarotation. Forms an osazone soluble in hot water. Hydrolysed by acids and more readily by maltase to two molecules of dextrose. Fermented after inversion by yeast enzymes.
Lactose	Milk of mammals.	+52.5°	Less soluble than maltose and forms mucic acid on oxidation, otherwise very similar. Hydrolysed by lactase to dextrose and galactose. Not fermented by ordinary yeast.
Melibiose.	Raffinose, by the action of acids, or top yeast.	+143°	Mutarotates. Hydrolysed by melibiase to dextrose and galactose. Fermented by bottom but not by top yeasts.
Turanose.	Melecitose, by the action of acids.	+71.8°	Hydrolysed by acids to dextrose and fructose. Forms a phenyl-osazone. Not hydrolysed by invertase.
Tri-saccharide. *Non-reducing*, $C_{18}H_{32}O_{16}$. Raffinose.	Sugar beet.	+104°	Does not form a hydrazone or osazone nor mutarotate. Fermented after hydrolysis. Converted into sucrose and galactose by emulsin, and into lævulose and melibiose by invertase.

actions as reducing agents, and their relations to polarised light. A third mode of determination is based on the sp. gr. of the saccharine solution. Other useful processes for estimation or differentiation are based on the behaviour of the sugars with yeast, and on treatment with concentrated and dilute acids.

Phenylhydrazine is of great value as a qualitative reagent and the asymmetrically disubstituted hydrazines may in some cases be used with advantage. When dealing with sugar solutions it is important to avoid carefully the presence of alkali. The general methods will be described in the following sections, before dealing with the special application of these and other processes to the examination of particular sugars or saccharine substances.

Specific Gravity of Saccharine Solutions.—Solutions of equal strengths containing different carbohydrates have approximately the same, though not strictly identical, sp. gr. In other words, the sp. gr. of the solution depends chiefly on the amount of solid dissolved.

The following table shows the sp. gr. of carbohydrate solutions under three different conditions. The figures refer in all cases to 15.5° (60° F.), water at the same temperature being taken as 1000.

Substance in solution	Formula	Specific gravity of solutions containing			Observer
		a 4.21% of carbo-hydrate.	*b* 10 grm. solid per 100 grm.	*c* 10 grm. solid per 100 c.c.	
Dextrose	C₆H₁₂O₆	1042.1	1040.0	1038.5	F. Salomon.
Starch glucose..........	....	1042.0	1039.9	1038.4	A. H. Allen.
Invert sugar........	2C₆H₁₂O₆ {	1042.4	1040.3	1038.8	G. H. and R.[1]
		1042.1	1040.0	1038.5	A. H. Allen. -
	(	1042.3	1040.2	1038.7	Chancel. -
Milk sugar..............	C₁₂H₂₂O₁₁	1040.6	1040.6	1039.1	O. Hehner.
	(	1040.6	1040.6	1039.0	G. H. and R.[1]
Cane sugar.............	C₁₂H₂₂O₁₁ {	1040.3	1040.3	....	Brix; Gerlach.
	(	1040.1	1040.1	1038.6	Brown and Heron.[2]
Maltose	C₁₂H₂₂O₁₁ {	1040.8	1040.8	1039.3	Brown and Heron.[2]
		1041.1	1041.1	1039.5	O'Sullivan, 1879.
Malt extract...........	.... {	....	1038.9	1037.5	Chas. Graham.
		....	1040.4	1038.9	Muspratt.
" pale........	....	1041.2	....	....	G. H. and R.[1]
" brown......	....	1041.2	....	....	G. H. and R.[1]
Dextrin	xC₁₂H₂₀O₁₀ {	1039.0	1041.1	1039.5	O'Sullivan, 1879.
		1039.2	1041.0	1039.4	H. T. Brown, 1884.
Starch paste...........	yC₁₂H₂₀O₁₀	1039.1	1041.3	1039.7	Brown and Heron.[2]
Caramel..............	C₁₂H₁₈O₉(?)	1034.9	1039.0	1037.5	G. H. and R.[1]

1. Report on Original Gravities, 1852 by Graham, Hofmann and Redwood.
2. *Trans. Chem. Soc.*, 1879, 35, 569.

In practice it is convenient to assume the solution densities of the carbohydrates to be uniformly 1.0386 for a concentration of 10 grm. per 100 c.c. whence the sp. gr. of the sugar in a state of solution is 1.628. It follows that the sum of the carbohydrates present in an aqueous solution may be found approximately by allowing an increase of 3.86 in density for each 1 grm. of carbohydrate in 100 c.c. of the liquid. This figure is correct for very dilute solutions, but for those containing more than 12 grms. of solids the divisor 3.85 gives closer results.[1]

$W = \frac{D - 1000}{3.85}$ where W=grm. of solids in 100 c.c. and D=the sp. gr. of the solution at 60° F. or $W = \frac{1000}{D} \cdot \frac{W}{}$ where w = weight of solids in 100 *parts by weight* of the liquid.

The foregoing formula is applicable for carbohydrate solutions of moderate strength; in case the sample is too dense to determine the density directly a weighed portion of it must first be diluted with a weighed quantity of water, or a weighed portion must be dissolved and diluted to a known volume of water. In the first instance:

Percentage of solids in the undiluted material = $\frac{WS}{w}$ where S = percentage of solids in the diluted material, W = weight of the diluted material, w = weight of the sample taken for dilution.

When the dilution is made to a definite volume:

Percentage of solids in the undiluted material=VDS/w, where V= volume of the diluted solution, D = sp. gr. of the diluted solution, S = percentage of solids in the diluted solution, w = weight of the sample taken for dilution.

In breweries it is often convenient to ascertain the sp. gr. of the wort at a temperature above that of 60° F. (=15.5°), in which case the sp. gr. as observed by the hydrometer can be calculated into the corresponding number for a temperature of 60° F. in the following manner:

To unity add .004 for every degree sp. gr. above 1000 (g) shown by the hot wort, and .01 for each Fahrenheit degree of temperature (t) above 60° F. Multiply the sum of these by 1/10 of the number of Fahrenheit degrees above 60° F., when the product, added to the sp. gr. of the hot wort, will be a number representing the sp. gr. of the liquid at 60° F. The rule is expressed by the following formula:

$$G = \left(1 + \frac{(g - 1000)4}{1000} + \frac{t - 60}{100}\right)\frac{t - 60}{10} + g$$

[1] Brown and Heron (*Trans. Chem. Soc.*, 1879, 35, 644) have laid down a curve by which the strength of cane-sugar solutions can be readily ascertained in all cases of less density than 1150.

Thus, if the wort be found to have a sp. gr. of 1052.0 at a temperature of 110° F., then by the formula:

$$G = \left(1 + \frac{(1052 - 1000)4}{1000} + \frac{110 - 60}{100}\right)\frac{110 - 60}{10} + 1052$$

$$G = (1 + .208 + .5)5 + 1052 = 1060.54$$

Saccharometers.—Various modifications of the hydrometer have been devised and used for ascertaining the sp. gr. of saccharine solutions.

Bates' brewers' saccharometer is much used for testing the strength of beer-worts, and hence it is described under "Malt."

On the Continent, Balling's saccharometer is much used. If B=degrees of Balling and b those of Bates, the indications of one instrument may be calculated to those of the other by the following formulæ:

$$B = \frac{260b}{360 + b} \; ; \text{ and } b = \frac{360B}{260 - B}$$

The saccharometer of Brix is practically the same as that of Balling. In each, the number of degrees is identical with the percentage by weight of cane sugar in the solution.

The Brix spindle should be graduated to tenths. It is therefore desirable, for accuracy, that the range of degrees recorded by each individual spindle be as limited as possible, this end being best secured by the employment of sets consisting of not less than three spindles. The solutions should be as nearly as possible of the same temperature as the air at the time of reading, and if the variation from the temperatures of the graduation of the spindle amount to more than 1°, compensation must be made by reference to the table of corrections for temperature, page 293. This temperature should be 17.5° C. Before taking the sp. gr. of a juice, it should be allowed to stand in the cylinder until all air bubbles have escaped.

A TABLE FOR THE COMPARISON OF SPECIFIC GRAVITIES, DEGREES BRIX AND DEGREES BAUME.

Degree Brix or per cent. by weight of sucrose	Specific gravity	Degree Baumé	Degree Brix or percent. by weight of sucrose	Specific gravity	Degree Baumé	Degree Brix or percent. by weight of sucrose	Specific gravity	Degree Baumé
1.0	1.00388	0.6	33.0	1.14423	18.5	65.0	1.31989	35.6
2.0	1.00779	1.1	34.0	1.14915	19.05	66.0	1.32601	36.1
3.0	1.01173	1.7	35.0	1.15411	19.6	67.0	1.33217	36.6
4.0	1.01570	2.3	36.0	1.15911	20.1	68.0	1.33836	37.1
5.0	1.01970	2.8	37.0	1.16413	20.7	69.0	1.34460	37.6
6.0	1.02373	3.4	38.0	1.16920	21.2	70.0	1.35088	38.1
7.0	1.02779	4.0	39.0	1.17430	21.8	71.0	1.35720	38.6
8.0	1.03187	4.5	40.0	1.17943	22.3	72.0	1.36355	39.1
9.0	1.03599	5.1	41.0	1.18460	22.9	73.0	1.36995	39.6
10.0	1.04014	5.7	42.0	1.18981	23.4	74.0	1.37639	40.1
11.0	1.04431	6.2	43.0	1.19505	23.95	75.0	1.38287	40.6
12.0	1.04852	6.8	44.0	1.20033	24.5	76.0	1.38939	41.1
13.0	1.05276	7.4	45.0	1.20565	25.0	77.0	1.39595	41.6
14.0	1.05703	7.9	46.0	1.21100	25.6	78.0	1.40254	42.1
15.0	1.06133	8.5	47.0	1.21639	26.1	79.0	1.40918	42.6
16.0	1.06566	9.0	48.0	1.22182	26.6	80.0	1.41586	43.1
17.0	1.07002	9.6	49.0	1.22728	27.2	81.0	1.42258	43.6
18.0	1.07441	10.1	50.0	1.23278	27.7	82.0	1.42934	44.1
19.0	1.07884	10.7	51.0	1.23832	28.2	83.0	1.43614	44.6
20.0	1.08329	11.3	52.0	1.24390	28.8	84.0	1.44298	45.1
21.0	1.08778	11.8	53.0	1.24951	29.3	85.0	1.44986	45.5
22.0	1.09231	12.4	54.0	1.25517	29.8	86.0	1.45678	46.0
23.0	1.09686	13.0	55.0	1.26086	30.4	87.0	1.46374	46.5
24.0	1.10145	13.5	56.0	1.26658	30.9	88.0	1.47074	47.0
25.0	1.10607	14.1	57.0	1.27235	31.4	89.0	1.47778	47.45
26.0	1.11072	14.6	58.0	1.27816	31.9	90.0	1.48486	47.9
27.0	1.11541	15.2	59.0	1.28400	32.5	91.0	1.49199	48.5
28.0	1.12013	15.7	60.0	1.28989	33.0	92.0	1.49915	48.9
29.0	1.12488	16.3	61.0	1.29581	33.5	93.0	1.50635	49.4
30.0	1.12967	16.8	62.0	1.30177	34.0	94.0	1.51359	49.8
31.0	1.13449	17.4	63.0	1.30777	34.5	95.0	1.52087	50.3
32.0	1.13934	17.95	64.0	1.31381	35.1			

The degrees of this table are apparently those of the Gerlach scale:

$$\text{Sp. gr.} = \frac{146.8}{146.8 - n}$$

If the determination be made at any other temperature than 17.5°, the result should be corrected by the use of the following table:

TABLE FOR CORRECTION OF THE READINGS OF THE BRIX SPINDLE WHEN THE READING IS MADE AT OTHER THAN THE STANDARD TEMPERATURE, 17.5°.

(For temperatures below 17.5° the correction is to be subtracted.)

Temperature	Degree Brix of the Solution												
	0	5	10	15	20	25	30	35	40	50	60	70	75
°C.													
0	0.17	0.30	0.41	0.52	0.62	0.72	0.82	0.92	0.98	1.11	1.22	1.25	1.29
5	0.23	0.30	0.37	0.44	0.52	0.59	0.65	0.72	0.75	0.80	0.88	0.91	0.94
10	0.20	0.26	0.29	0.33	0.36	0.39	0.42	0.45	0.48	0.50	0.54	0.58	0.61
11	0.18	0.23	0.26	0.28	0.31	0.34	0.36	0.39	0.41	0.43	0.47	0.50	0.53
12	0.16	0.20	0.22	0.24	0.26	0.29	0.31	0.33	0.34	0.36	0.40	0.42	0.46
13	0.14	0.18	0.19	0.21	0.22	0.24	0.26	0.27	0.28	0.29	0.33	0.35	0.39
14	0.12	0.15	0.16	0.17	0.18	0.19	0.21	0.22	0.22	0.23	0.26	0.28	0.32
15	0.09	0.11	0.12	0.14	0.14	0.15	0.16	0.17	0.16	0.17	0.19	0.21	0.25
16	0.06	0.07	0.08	0.09	0.10	0.10	0.11	0.12	0.12	0.12	0.14	0.16	0.18
17	0.02	0.02	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.06
18	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02
19	0.06	0.08	0.08	0.09	0.09	0.10	0.10	0.10	0.10	0.10	0.10	0.08	0.06
20	0.11	0.14	0.15	0.17	0.17	0.18	0.18	0.18	0.19	0.19	0.18	0.15	0.11
21	0.16	0.20	0.22	0.24	0.24	0.25	0.25	0.25	0.26	0.26	0.25	0.22	0.18
22	0.21	0.26	0.29	0.31	0.31	0.32	0.32	0.32	0.33	0.34	0.32	0.29	0.25
23	0.27	0.32	0.35	0.37	0.38	0.39	0.39	0.39	0.40	0.42	0.39	0.36	0.33
24	0.32	0.38	0.41	0.43	0.44	0.46	0.46	0.47	0.47	0.50	0.46	0.43	0.40
25	0.37	0.44	0.47	0.49	0.51	0.53	0.54	0.55	0.55	0.58	0.54	0.51	0.48
26	0.43	0.50	0.54	0.56	0.58	0.60	0.61	0.62	0.52	0.66	0.62	0.58	0.55
27	0.49	0.57	0.61	0.63	0.65	0.68	0.68	0.69	0.70	0.74	0.70	0.65	0.62
28	0.56	0.64	0.68	0.70	0.72	0.76	0.76	0.78	0.78	0.82	0.78	0.72	0.70
29	0.63	0.71	0.75	0.78	0.79	0.84	0.84	0.86	0.86	0.90	0.86	0.80	0.78
30	0.70	0.78	0.82	0.87	0.87	0.92	0.92	0.94	0.94	0.98	0.94	0.88	0.86
35	1.10	1.17	1.22	1.24	1.30	1.32	1.33	1.35	1.36	1.39	1.34	1.27	1.25
40	1.50	1.61	1.67	1.71	1.73	1.79	1.79	1.80	1.82	1.83	1.78	1.69	1.65
50	....	2.65	2.71	2.74	2.78	2.80	2.80	2.80	2.80	2.79	3.70	2.56	2.51
60	....	3.87	3.88	3.88	3.88	3.88	3.88	3.88	3.90	3.82	2.70	3.43	3.41
70	....	5.17	5.18	5.20	5.14	5.13	5.10	5.08	5.06	5.06	4.90	4.72	4.47	4.35
80	....	....	6.62	6.59	6.54	6.46	6.38	6.30	6.26	6.06	5.82	5.50	5.33
90	....	....	8.26	8.16	8.06	7.97	7.83	7.71	7.58	7.30	6.96	6.58	6.37
100	....	....	10.01	9.87	9.72	9.56	9.39	9.21	9.03	8.64	8.22	7.76	7.42

Example.—A sugar solution shows a reading of 30.2° Brix at 30°. To find the necessary correction for the conversion of this reading to the reading which would have been obtained if the observation had been made at 17.5°., find the vertical column in the table headed 30° Brix, which is the nearest to the observed reading. Follow down this column until the number is reached which is opposite to the temperature of observation—in this case 30°. The number found, 0.92, is to be added to the observed reading.

Mohr (*Zeit. Spiritusind*, 1906, 29, 25) has recalculated and co-ordinated the existing data relating to the sp. gr. of solutions of the different sugars. He shows the percentage by weight and the concentration in grm. per 100 c.c. for each of the sugars with the corresponding sp. grs. of sucrose solutions of the same concentration at the same temperature and the percentage by weight of cane sugar in solutions of the same sp. grs.

ANHYDROUS DEXTROSE (Salomon, *Ber.*, 1881, 14, 2711).

Contents of solution. Grm. per 100 c.c.	Contents of solution. % by weight	Sp. gr. of dextrose solution at 17.5°/17.5°	Sp. gr. of cane-sugar solution of same concentration at 17.5°/17.5°	Contents of cane-sugar solution of same sp. gr. as dextrose. % by weight.
1.00	0.998	1.00375	1.00387	0.967
2.00	1.988	1.0075	1.00774	1.926
3.00	2.970	1.0115	1.01160	2.944
4.00	3.945	1.0153	1.01547	3.903
5.00	4.912	1.0192	1.01933	4.880
6.00	5.873	1.0230	1.02319	5.825
7.00	6.827	1.0267	1.02705	6.740
8.00	7.773	1.0305	1.03089	7.677
9.00	8.714	1.0342	1.03475	8.581
10.00	9.645	1.0381	1.03858	9.527
11.00	10.570	1.0420	1.04243	10.467
12.00	11.490	1.0457	1.04628	11.352
13.00	12.402	1.0495	1.05011	12.257
14.00	13.309	1.0533	1.05396	13.155
15.00	14.209	1.0571	1.05780	14.047
16.00	15.100	1.0610	1.06161	14.958
17.00	15.984	1.0649	1.06542	15.863
18.00	16.864	1.0687	1.06924	16.740

LÆVULOSE (Ost., Lippman's "Chem. der Zuckerarten," 3d Edition, 1, 819).

Contents of solution. Grm. per 100 c.c.	Contents of solution. % by weight.	Sp. gr. of a lævulose solution at 20°/4°	Sp. gr. of cane-sugar solution of same concentration at 20°/4°	Contents of cane-sugar solution of same sp. gr. as lævulose. % by weight.
1.01	1.0100	1.0021	1.00216	0.995
1.03	1.0324	1.0022	1.00224	1.002
2.01	1.9949	1.0062	1.00600	2.047
2.04	2.0263	1.0063	1.00612	2.073
5.03	4.9395	1.0177	1.01761	4.961
5.04	4.9575	1.0178	1.01768	4.986
5.06	4.9710	1.0178	1.01773	4.986
8.04	7.8051	1.0295	1.02915	7.891
9.28	8.9724	1.0341	1.03392	9.018
10.19	9.8195	1.0379	1.03740	9.941
10.95	10.5199	1.0405	1.04029	10.570
19.90	18.5161	1.0748	1.07441	18.605
21.93	20.2638	1.0821	1.08213	20.257
33.56	29.7995	1.1263	1.12603	29.857
33.97	30.1157	1.1279	1.12754	30.193

INVERT SUGAR (Herzfeld, *Z. Ver. deut. Zuckerind*, 37, 912).

Contents of solution. Grm. per 100 c.c.	Contents of solution % by weight	Sp. gr. of invert sugar solution.		Sp. gr. of cane-sugar solution of same concentration at 17.5°/17.5°	Contents of cane-sugar solution of same sp. gr. as invert sugar. % by weight.
		At 17.5°/4°	At 17.5°/17.5°		
10.39	10.0	1.03901	1.04034	1.04005	10.07
10.93	10.5	1.04109	1.04243	1.04214	10.57
11.47	11.0	1.04316	1.04450	1.04422	11.07
12.02	11.5	1.04527	1.04661	1.04632	11.57
12.57	12.0	1.04737	1.04871	1.04841	12.07
13.12	12.5	1.04949	1.05083	1.05053	12.57
13.67	13.0	1.05160	1.05295	1.05264	13.07
14.78	14.0	1.05588	1.05723	1.05690	14.08
15.90	15.0	1.06018	1.06154	1.06118	15.08
17.03	16.0	1.06453	1.06590	1.06549	16.09
18.17	17.0	1.06889	1.07026	1.06983	17.10
19.32	18.0	1.07330	1.07468	1.07421	18.11
20.48	19.0	1.07772	1.07912	1.07862	19.11
21.64	20.0	1.08218	1.08357	1.08306	20.11
22.82	21.0	1.08665	1.08804	1.08753	21.11
24.00	22.0	1.09114	1.09254	1.09203	22.11
25.20	23.0	1.09566	1.09707	1.09658	23.11
26.40	24.0	1.10019	1.10160	1.10115	24.10
27.62	25.0	1.10474	1.10616	1.10575	25.09
28.84	26.0	1.10930	1.11072	1.11039	26.07
30.09	27.0	1.11433	1.11576	1.11506	27.15

ANHYDROUS MALTOSE (Salomon. *J. prakt. Chem.*, [2], 28, 82).

Contents of solution. Grm. per 100 c.c.	Contents of solution. % by weight.	Sp. gr. of maltose solution at 17.5°/17.5°	Sp. gr. of cane-sugar solution of same concentration at 17.5°/17.5°	Contents of cane-sugar solution of same sp. gr. as maltose. % by weight.
1.0	0.997	1.00393	1.00387	1.013
2.0	1.987	1.00785	1.00774	2.015
3.0	2.969	1.01177	1.01160	3.013
4.0	3.943	1.01568	1.01546	3.998
5.0	4.911	1.01953	1.01932	4.963
6.0	5.870	1.02340	1.02318	5.925
7.0	6.823	1.02733	1.02703	6.895
8.0	7.768	1.03122	1.03087	7.855
9.0	8.706	1.03515	1.03471	8.812
10.0	9.637	1.03900	1.03855	9.746
15.0	14.192	1.05827	1.05773	14.321
20.0	18.587	1.07740	1.07680	18.723
25.0	22.829	1.09650	1.09580	22.982
30.0	26.928	1.11550	1.11472	27.096

Lævulose, invert sugar, and maltose solutions have higher sp. gr. than the corresponding solutions of cane sugar, whilst those of dextrose have lower sp. gr. It will be seen that the use of the cane-sugar tables for the determination of other sugars involves an error of only 0.1 %.

Action of Strong Acids on Sugars.—Organic acids act on sugars to form oxygen esters. In presence of suitable dehydrating agents acetic acid or acetic anhydride gives rise to fully acetylated compounds, viz., the pentacetate in the case of the glucoses or the octacetate of sucrose, maltose, or lactose. Dextrose pentabenzoate may be used for the detection and isolation of dextrose, particularly in physiological fluids. The solution is shaken for an hour with six parts of benzoyl chloride and 48 parts of 18 to 20% sodium hydroxide for every part of dextrose and cooled with ice. After 24 hours the pentabenzoate may be recrystallised from alcohol. It forms colourless needles; m. p., 179°.

Nitric acid when used in cold concentrated solution gives rise to nitric esters. When heated with dilute or moderately concentrated acid the sugars yield oxidation products, of which mucic, saccharic, tartaric and racemic acids are the most constant and characteristic. The formation of mucic acid is characteristic of galactose and also of di- or polysaccharides which contain galactose, e. g., milk sugar or gums. For the estimation of galactose as mucic acid, see under Galactose, p. 376.

Sulphuric Acid.—Dextrose dissolves in cold concentrated sulphuric acid without any colouration, forming dextrosesulphonic acid. This behaviour distinguishes dextrose from cane sugar, which is carbonised by concentrated sulphuric acid with great facility. A strong syrup of cane sugar mixed with concentrated sulphuric acid is immediately decomposed with evolution of sulphur dioxide and other volatile products, and formation of a bulky, black, carbonaceous mass.

Action of Dilute Acids on Sugars. Hydrolysis or Inversion.— When an aqueous solution of cane sugar is heated with dilute sulphuric or hydrochloric acid, the solution increases in s_p. gr., and the sugar loses its power of crystallising readily. This change in properties is attended by the assimilation of the elements of water, with formation of the mixture of dextrose and lævulose known as inverted or invert sugar: $C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$. The rate of hydrolysis depends mainly on the proportion of acid used, its chemical activity, and the temperature employed in the operation. When cane sugar is hydrolysed the optical activity is changed from right- to left-handed or is "inverted." The term *inversion* is often applied generally to the process of hydrolysis of the di-saccharides whether or not the same optical change be produced.

The property of undergoing hydrolysis when heated with dilute

acids is common to all the di- and poly-saccharides. The following table shows the products of hydrolysis of the principal di- and tri-saccharides:

Di-saccharide.	=	*Mono-saccharides.*
Cane sugar	=	dextrose and lævulose.
Milk sugar	=	dextrose and galactose.
Maltose	=	dextrose.
Melibiose	=	dextrose and galactose.
Raffinose	=	lævulose, dextrose and galactose.

Sucrose is most readily and certainly inverted by adding to a solution containing not more than 25 grm. of the solid per 100 c.c. one-tenth of its bulk of fuming hydrochloric acid, and then heating the liquid to 70° for 10 or 15 minutes. Some operators prefer dilute sulphuric to hydrochloric acid, and heat the liquid to boiling for 5 or 10 minutes.

Lactose is less readily hydrolysed than sucrose, being unaffected by boiling for ten minutes with 2 grm. of citric acid per 100 c.c. of the solution.

The differences in the readiness with which different sugars are hydrolysed is shown in the following table[1] in which the rate of hydrolysis by hydrochloric acid at about 70° of a number of substances under identical conditions is recorded relatively to the most stable compound which is expressed as 100.

*a*Methyl-glucoside,	100
*β*Methyl-glucoside,	180
*a*Methyl-galactoside,	540
*β*Methyl-galactoside,	880
Salicin,	600
Lactose,	720
Maltose,	740
Cane sugar,	about 900,000

To insure complete hydrolysis of carbohydrates other than cane sugar, dilute solutions—preferably not above 5 %—should be employed and the heating prolonged. Meissel (*Z. anal. chem.*, **22**, 115) uses 3 % sulphuric acid or 5 % fuming hydrochloric acid, obtaining in the former case a conversion of 98.5 %. The liquid is heated in a water-bath for 3 or 4 hours. O'Sullivan states that the purest yield of dex

[1] E. F. Armstrong *Proc. Roy. Soc.* 1904, **74**, 188–194.

trose is obtained by heating 30 grm. of the saccharine matter in 100 c.c. of 1 % sulphuric acid at a pressure of one additional atmosphere; pure dextrose results after 20 minutes' treatment. When the inverted solution of a sugar is to be decolourised by basic lead acetate or treated by Fehling's solution, the free acid contained in it should first be *nearly* neutralised by the addition of sodium carbonate.

Action of Alkalies on Sugars.—Cane sugar is not attacked by dilute caustic alkalies or alkaline carbonates in the cold, and only very slowly, if at all, on heating. It is decomposed by boiling with concentrated alkaline solutions, and when fused with potassium hydroxide yields potassium oxalate and acetate and other products. Cane sugar forms a few well-established compounds with bases and many with salts.

Dextrose and other mono-saccharides are readily decomposed by alkalies. When heated with sodium or potassium hydroxide, dextrose becomes brown at 60-70°, and decomposes entirely on prolonged boiling.

Fermentation of Sugars.—Dextrose, fructose, mannose, and invert sugar are fermentable by all yeasts.[1] Cane sugar, maltose, lactose, melibiose and raffinose are fermentable only after inversion by dilute acids or by an appropriate enzyme. Ordinary yeast, *Saccharomyces cerevisiæ*, contains the enzymes which hydrolyse cane sugar and maltose and can ferment both these sugars. Fermentation is preceded by inversion and, indeed, if the proportion of yeast is very small, the change of sucrose does not go beyond the formation of invert sugar.

To recognise the presence of a fermentable sugar by means of yeast, care must be taken (1) that the aqueous solution is not too concentrated (from 5 to 10 % is the most suitable concentration); (2) that the liquid is neutral or faintly acid, alkalinity being carefully neutralised; (3) that the liquid is wholly free from antiseptics of any kind which would prevent the alcoholic fermentation.

The yeast should be fresh, free from starch and carefully washed with a little cold distilled water before use.

5 to 10 c.c. of the liquid are mixed with a little yeast, placed in a test-tube closed with a plug of sterilised cotton wool, and incubated at 20-30° for a few hours. In a positive experiment the signs of fermentation are unmistakable, and gentle shaking will cause the bubbles of gas to rise to the surface. It is always desirable to make a blank

[1] See E. F. Armstrong, *Proc. Roy. Soc.*, 1905, 76B, 600.

experiment so as to ascertain positively that the yeast does not itself yield any notable quantity of carbon dioxide under the conditions of the experiment.

The foregoing process may readily be made roughly quantitative by attaching a delivery tube and collecting the gas formed over mercury. More accurate results are obtained by using an apparatus such as is employed for the analysis of carbonates and determining the loss of weight during fermentation. The dissolved carbon dioxide may be swept out by a current of air before the final weighing. The fermentation should be continued as long as any notable quantity of gas continues to be evolved. The weight of carbon dioxide multiplied by 2.0454 gives that of the dextrose fermented, which figure multiplied by 0.95 gives the corresponding weight of sucrose or.maltose.

Instead of measuring or weighing the carbon dioxide produced it is in some respects preferable to estimate the *alcohol* formed. The process is conducted as already described, but it is not desirable to employ less than 50 or 100 c.c. of the solution, which should by preference have a concentration of 12 to 16 per cent.; 0.5 grm. of pressed fresh yeast is sufficient in most cases, especially if a little yeast-ash be added, but it is desirable to add a little more yeast at the end of the action to insure that no further fermentation can be induced. The liquid should be kept at a temperature of 20° to 25° for 2 or 3 days, after which the liquid is distilled to about 1/3, the distillate weighed, and the alcohol contained in it ascertained from the sp. gr. The weight of alcohol thus found when multiplied by 2.02 gives the dextrose or by 1.96 the sucrose from which it was derived.

Some operators prefer to employ a large quantity of yeast, such as 10 or even 20 grm. In such cases it is very desirable to conduct a blank experiment with the same quantity of yeast and water, side by side with the test of the saccharine liquid, and to deduct the alcohol found in the former case from that obtained in the latter before calculating to the equivalent of sugar. A still better plan, perhaps, is to ferment a solution of sucrose or invert sugar, of known strength, side by side with the samples, when the amounts of sugar in the two liquids will bear to each other the same proportion as the amounts of alcohol produced by their distillation.

Another method which has been suggested for estimating sugar from the results of its fermentation by yeast consists in noting the "gravity lost" in the process; that is, the sp. gr. of the original saccha-

rine solution is observed and compared with that of the fermented liquid, after filtering, washing the residue, boiling off the alcohol, and making up the solution to its original volume. The difference is the "gravity lost" by the fermentation. The "spirit indication" corresponding to the value thus found is ascertained by reference to the table on page 153, and this figure subtracted from 1000 gives the density of the dilute alcohol produced by the fermentation. The strength of this can be ascertained by reference to the tables, and the weight so arrived at can be calculated into its equivalent of sucrose or maltose by the factor 1.96, or into glucose by the factor 2.02. The dextrose may also be deduced by calculating 0.219 % for each degree of gravity lost.

It is evident that the last-described method can be advantageously employed as a check on the distillation process.

Instead of estimating the sugar from the sp. gr. of the solution before and after fermentation, equal volumes of the original and the filtered fermented liquids may be evaporated to dryness, and the quantity of sugar deduced from the loss of weight. An addition of 5 % to the amount of sugar thus found should be made as a correction for the succinic acid and glycerol which are produced by the fermentation and remain in the residue from the fermented liquid. When the quantity of sugar is small, this method is preferable to an estimate based on the gravity lost.

In determining sugar by fermentation with yeast it is desirable to add to the solution a little yeast-ash or sodium phosphate and potassium nitrate, so as to furnish the yeast with the inorganic elements requisite for its nutrition.

The estimation of sugar by fermentation with yeast is occasionally very valuable, and when the process is carefully conducted the results are fairly accurate.

The application of pure culture yeasts to the separation of different sugars promises valuable results. The sugar not attacked by the yeast in a mixture of fermentable and unfermentable carbohydrate can be estimated accurately; the estimation of the quantity of the fermented sugar from the carbon dioxide lost does not always give trustworthy results.

The quantitative fermentation of sugars requires from 6 to 7 days or longer if maltose is to be fermented in presence of dextrin. The use of yeast affords practically the only accurate method of separating dextrose from maltose in the analysis of starch syrups.

	Dextrose $C_6H_{12}O_6$	Lævulose $C_6H_{12}O_6$	Milk sugar $C_{12}H_{22}O_{11}+H_2O$	Maltose $C_{12}H_{22}O_{11}+H_2O$	Cane sugar $C_{12}H_{22}O_{11}$	Dextrin $C_6H_{10}O_5$
1. Moisten the solid sugar with water, and stir in the cold with concentrated sulphuric acid (1.845 sp. gr.).	Not affected when pure.	Not affected when pure	Not affected.	Slightly reddish or brownish, gradually turning darker	Charred.	Not affected.
2. Triturate the solid sugar with sodium hydroxide, or boil it with a 3% solution for one minute.	Deep brown colouration.	Deep brown colouration.	Not affected.	Slightly discoloured.	Not affected.	No change.
3. To the neutral aqueous solution add a few drops of Fehling's solution and heat to boiling for a few minutes.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	No change.	No change.
4. ¹⁄₂₀ of its neutralise heat to	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.
5. Heat the solution in boiling water for 3 minutes with 2 c.c. of a liquid containing : and 1% of acetic	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	No change.	No change	No change.	No change.
6. n the polarimeter.	Dextro-rotatory.	Levo-rotatory.	Dextro-rotatory.	Dextro-rotatory.	Dextro-rotatory.	Dextro-rotatory.
7. Heat solution with dilute acid as in test 4, and observe again in polarimeter.	Dextro-rotatory power unchanged.	Levo-rotatory power unchanged.	Dextro-rotatory power increased.	Dextro-rotatory power diminished.	Dextro-rotatory power changed to levo-rotatory.	Dextro-rotatory power diminished.
8. Heat the solution in boiling water for an hour with 1 c.c. of phenylhydrazine and 1 c.c. of 50% acetic acid and a little salt. Allow to cool (see page 398).	Yellow crystalline osazone insoluble in boiling water.	Same as dextrose.	Yellow crystalline osazone soluble in hot water.	Yellow crystalline osazone soluble in hot water.	No change.	Very soluble yellow osazones.
9. Treat the sugar with moderately concentrated nitric acid, and study the products of the oxidation (see page 296).	Saccharic acid.	Saccharic acid.	Mucic, saccharic and oxalic acids.	Saccharic and oxalic acids.	Saccharic and oxalic acids.	Oxalic acid.

Recognition of the Principal Kinds of Sugar.—When a sugar has been isolated in a condition of tolerable purity, it may be recognised by the special characters described in the tables of properties on page 301. The detection or identification of a sugar by its reactions is greatly simplified by applying the tests in a systematic manner.

All the substances referred to in the table are optically active. Hence it is not possible to have an inactive solution containing a notable quantity of one of the above sugars. If lævulose is present, together with a certain proportion of one of the other sugars, the solution may exhibit no rotation at a certain temperature, but would do so on heating or cooling, owing to the marked influence of temperature on the reduced optical activity of lævulose.

Lævulose always occurs in practice in presence of more or less dextrose, and in such cases is most easily detected by the change in the optical activity of the solution on heating. Other distinctions between lævulose and dextrose will be found under "Lævulose."

Milk sugar is only met with in products derived from milk. It is peculiar in having its optical activity and cupric reducing power increased by treatment with dilute acid, and in yielding mucic acid on oxidation with nitric acid. Physically it is distinguished from other sugars by its crystalline form and sparing solubility in cold water.

Cane sugar is well characterised by its behaviour towards invertase in addition to tests 1, 3, 4, 5, and 6.

Maltose when unmixed with dextrose is distinguished from the latter by reactions 2 and 5, but if dextrose be also present only a quantitative application of tests 3, 4, 5, and 6 will suffice for the detection of maltose.

Dextrin, which often occurs together with maltose, may be detected in mixtures of the two by gradually adding a large excess of strong alcohol, when it is precipitated in flocks which often adhere to the sides of the beaker as a gummy mass. Dextrin is said to be unaffected in its optical activity by boiling with a concentrated alkaline solution of mercuric cyanide, by which treatment maltose and dextrose are oxidised and destroyed.

Numerous colour reactions for the identification of the more important sugars in carbohydrate mixtures have been described, most of which have the disadvantage that they are not very characteristic. A discussion of these hardly enters into the subject of commercial analysis. Fenton's test for carbohydrates (*Proc. Camb. Phil. Soc.*, 1906, **14**, 24) which involves the formation of ω-bromomethylfurfuralde-

hyde, the merest trace of which may be detected, is of extreme delicacy. A small quantity of the sample is moistened with water, mixed with a few drops of phosphorus tribromide dissolved in toluene, and heated on a water-bath at 90–100° till it has become dark-coloured. It is then cooled, stirred with a few drops of ethyl malonate in a little alcohol and made alkaline by alcoholic potassium hydroxide. A characteristic blue fluorescence is obtained on dilution with much water.

m-Dinitrobenzene gives a violet co ouration with aldoses and ketoses in moderately alkaline solution. This appears usually after 15 minutes in a 1 % sugar solution.

For the estimation of sugars in admixture the cupric reducing power (K) and specific rotatory power $[a]_D$ should be ascertained under the following circumstances:

In the *original* solution of known concentration.

In the solution after treatment with *invertase*.

In the solution after heating for some hours with dilute sulphuric acid.

The following table shows the relative cupiic reducing power (K) (estimated gravimetrically) of the principal sugars, that of dextrose being taken as 100; and the specific rotatory power $[a]_D$ of the solutions of the original substances, and of the inverted solutions thereof, corrected for increase in volume. The values given are in all cases calculated for the *anhydrous* substance, and the volume of the solution is assumed to remain unchanged, any dilution being duly allowed for.

	Dex-trose	Lævu-lose	Milk sugar	Maltose	Cane sugar	Dextrin
Cupric Oxide Reducing Power (= K):						
a. Of *original* solution.	100	100	67.8	62	0	0
b. After treatment with *invertase*,	100	100	67.8	62	105.3	0
c. After heating with *acid*,	100	100	97.7	105.3	105.3	111.1
Specific Rotatory Power (for Sodium ray = $[a]_D$):						
a. For *original* solution,	+52.7	—98.8	+55.8	+139.2	+66.5	+198
b. After treatment with *invertase*,	+52.7	—98.8	+55.8	+139.2	—24.3	+198
c. After heating with *acid*,	+52.7	—98.8	+71.0	+ 55.0	—24.3	+ 58.5

It must be borne in mind that the figures representing the cupric reducing powers after treatment with invertase or dilute acid are not the values of K for the original weights of substance, but for the *products of the inversion.*

In determining the values of K and $[a]_D$, it is necessary to know the amount of sugar employed in the operation. This is best ascertained by evaporating to dryness a known measure of the solution employed for the analysis, but in some respects there is an advantage in calculating the strength of the solution from the sp. gr. The concentration of solutions of pure cane sugar can be accurately ascertained by dividing the excess of sp. gr. over that of water by 3.86, as described on page 290, but this divisor is not strictly accurate for solutions of other carbohydrates. In practice it is sometimes very convenient to follow the practice of Brown and Heron (*Jour. Chem. Soc.*, 1879, 35, 569), and assume all solutions of carbohydrates to have the sp. gr. of cane-sugar solutions of the same strength, using appropriately modified figures to express the values of K and $[a]_D$. As the true sp. gr. of a solution of dextrin containing 10 grm. of the dry solid in 100 c.c. measure is 1039.4 , the value of $[a]_{D\ 3.86}$ will be $198 \times \frac{3.86}{3.94} = +194$.

The following is a description in outline of the mode of procedure which should be adopted in the application of the foregoing principles to the analysis of one of the most complicated saccharine mixtures likely to be met with in practice. It assumes the presence in admixture of dextrose, lævulose, sucrose, maltose, dextrin, and gallisin, together with water and mineral matter. Such a mixture would be represented by honey which had been adulterated both with cane sugar and glucose syrup (see page 385).

The *total solids* are estimated by evaporating a known measure of the solution to dryness in a flat dish. On deducting the *ash* left on igniting the residue the amount of the *organic solids* will be ascertained. The solids may also be deduced from the density of the solution by dividing the excess above 1000 by 3.86 (see p. 290).

The *dextrin* may be precipitated by pouring the aqueous solution gradually into a large excess of rectified spirit. After standing till the precipitate is completely settled, the liquid is poured off and the dextrin estimated in the residue by direct weighing, or deduced from the solution-density or optical activity of the redissolved residue.

The *gallisin* may be estimated by distilling the alcoholic solution obtained in *b*, fermenting an aliquot part of the residual liquid, and treating the filtered solution left after complete fermentation by Fehling's solution.

The rotation due to the *sum* of the optically active bodies present is ascertained on the clarified solution at 15°.

The *lævulose* is estimated from the change in the rotatory power of the solution on heating.

The *sucrose* is estimated from the change produced in the rotatory power of the solution by treatment with invertase. The result is confirmed by the change in the cupric oxide reducing power of the solution caused by the action of the invertase.

The cupric reducing power of the original solution is determined gravimetrically by Fehling's solution. From the value for K thus obtained that due to any gallisin found after fermentation is deducted, and from the remainder is subtracted the reduction due to any lævulose present. The difference is the reducing power due to the dextrose and maltose. The sum of their weights having been ascertained by deducting the lævulose, sucrose, dextrin, gallisin, and ash from the total solids, the amounts of *maltose* may be calculated by subtracting the value of K for the two bodies from the sum of the percentages of the two, and dividing the difference by 0.38. The maltose thus found is subtracted from the sum when the percentage of *dextrose* will be arrived at.

Specific Rotatory Power of Sugars.—The principles used in the construction of polarimeters are defined in the Introduction to this volume. To determine the specific rotatory power of a carbohydrate the more scientific plan is to use an instrument graduated in circular degrees and observe the rotation caused by a known weight of the sugar dissolved in a known quantity of water; it is further necessary to know the temperature of the solution and the length of the tube. The alternative method is to make use of an instrument in which the deviation is produced by a plate of quartz 1 mm. in thickness for which that strength of sucrose which will produce the same deviation when examined in a 2-dcm. tube is determined. Such instruments are usually graduated so that the percentage of sugar present in the solution examined which contains the normal weight of the sample may be read off directly. For a full discussion of the large amount of work on this subject the reader is referred to special text-books, particularly that of Landolt—"Optical Rotatory Power."

The specific rotation of sugars is sensibly affected by the concentration of the solution and not always in the same direction. Thus strong solutions of sucrose cause a less deviation than the same amount of sugar would in more di'ute solutions, while with dextrose the reverse is the case.

The approximate value of $[a]_D$ for a solution of sucrose containing 10 grm. in 100 c.c. of liquid is +66.5°. Tollens gives the following formulæ for the exact apparent specific rotatory power, in which p represents the concentration of the sugar.

Solutions containing 18 to 69% sucrose.

$[a]_D^{20} = 66.386 + 0.015,035\ p - 0,000,3986\ p^2.$

So utions contain ng 4—18% sucrose.

$[a]_D^{20} = 66.810 - 0.015,553\ p - 0.000,052,462,\ p^2.$

There are very many difficulties in the way of obtaining a constant value for $[a]_j$, due in part to the fact that the transition tint is not a ray of definite refrangibility and even differs with different observers.

The value generally adopted is +73.8° whence $[a]_j / [a]_D = 1.110$ and $[a]_D / [a]_j = 0.9011$.

Brown and Millar (*Trans. Chem. Soc.*, 1897, **71**, 73) give the following factors for converting $[a]_D$ into $[a]_j$:

Sugar.	Per cent.	Factor.
Cane sugar,	10	1.107
Maltose,	10	1.113
Maltose,	5	1.111
Dextrose,	10	1.115
Dextrose,	5	1.111
Starch products,	10	1.111
Starch products,	5	1.111

The following table contains the most reliable observations of specific rotation of the more important species of sugar for solutions containing 10% or so of the solid sugar. The optical properties of the rarer sugars are shown in the tables on pages 287-8.

Sugar.	$[a]_D$.
d-Dextrose,	+52.7°
d-Galactose,	+81°
d-Lævulose,	—93.8°
Sucrose,	+66.5°
Maltose,	+138°
Lactose,	+52.5°
Raffinose,	+104°

Polarimetric Estimation of Sucrose in the Absence of Other Substances.—Until recently, although the general principles of the methods of optical saccharimetry were well understood, each ob-

server modified the more minute details of the process to suit his special convenience and in consequence slightly different results were habitually obtained in different countries. To obviate this, the International Commission for unifying methods of sugar analysis was called into being. At the Paris meeting in 1900, the normal temperature of +20° was adopted and all measuring vessels are required to be graduated in true metric c.c. at this temperature.

The preparation of the normal sugar solution is as follows: 26 grm. of chemically pure dry sugar,[1] weighed in air with brass weights, are dissolved in water at 20° in a flask graduated to contain 100 true c.c. (or 26.048 grm. of pure sugar in 100 Mohr c.c.) the solution filled up to the mark, well mixed, filtered if necessary, and polarised in a 200 mm. tube at 20° C. The apparatus must under these conditions indicate 100 units on the scale, and each scale division corresponds to 0.26 grm. of sucrose.

For laboratories in which temperatures are usually higher than 20°, it is permissible to graduate saccharimeters at any suitable temperature under the conditions specified above, providing that the analysis of the sugar be made at the same temperature—that is, that the volume be completed and the polarizations made at the temperature specified.

For the purposes of saccharimetry, it is found convenient in practice to employ a constant weight of each sample. The weight to be taken ranges from 16.19 to 26.07 grm., according to the instrument to be employed, and to a less degree with each particular instrument. With Soleil's saccharimeter the standard weight is 16.350 grm., and with other instruments, showing directly the percentage-content of real sugar in the sample, weights closely approximating to 16.337 grm. are usually employed. With polarimeters furnished with the Ventzke[2] scale, however, the standard weight is 26.048 grm. altered since 1900 to 26.0 grm.

For the assay of commercial sugar, the sugar scale divisions are the most convenient, but for the estimation of the percentage content of a carbohydrate in solution the use of circular degrees is preferable.

[1] To prepare pure sugar, further purify the purest commercial sugar in the following manner: Prepare a hot saturated aqueous solution, precipitate the sugar with absolute ethyl alcohol, spin the sugar carefully in a small centrifugal machine, and wash in the latter with absolute alcohol. Redissolve the sugar thus obtained in water, again precipitate the saturated solution with alcohol, and wash as above. Dry the second crop of crystals between blotting-paper and preserve in glass vessels for use. Determine the moisture still contained in the sugar and take this into account when weighing the sugar which is to be used.

[2] In the original Soleil-Ventzke instruments the scale was so divided that a solution of cane sugar, of a sp. gr. of 1.10 at 17°.5, observed in a tube 20 centimeters in length, rotated 100 divisions. A solution of sugar of the above sp. gr. is obtained by dissolving 26.048 grm. in water and diluting the liquid to 100 c.c.

The following table comparing the various instruments will be found useful:

Instrument	Normal weight of sugar	1 Sugar scale division = Angular degrees D	1 Angular degree D = divisions
German instruments, Schmidt and Haensch, Ventzke, Scheibler, etc. ...	26.048	0.3468	2.8835
Soleil-Duboscq	16.35	0.2175	4.597
Laurent	16.27	0.2167	4.6154
Wild	10.0	0.1331	0.7551

It is now recognised that a rise of temperature occasions a lowering of the rotation of sucrose. To correct for this Watts and Tempany give the formula-polarisation —0.00031 tN where N is the Ventzke scale reading and t the difference between the temperature of observation and that at which the instrument was standardised.

The following precise instructions regarding the care of the instruments used are given by the A. O. A. C.:

In effecting the polarisation of substances containing sugar employ only half-shade or triple field instruments.

During the observation keep the apparatus in a fixed position and so far removed from the source of light that the polarisation nicol is not warmed. Make several readings and take the mean thereof, but no one reading may be neglected.

In making a polarisation use the whole normal weight for 100 c.c., or a multiple thereof for any corresponding volume.

As clarifying and decolourising agents use either of basic lead, acetate, alumina cream, or concentrated solution of alum. Boneblack and similar decolourising agents are to be excluded.

After bringing the solution exactly to the mark at the proper temperature and after wiping out the neck of the flask with filter-paper, pour all of the well-shaken clarified sugar solution on a rapidly acting filter. Reject the first portions of the filtrate and use the rest, which must be perfectly clear for polarization.

PREPARATION OF REAGENTS.

(1) **Basic Lead Acetate Solution.**—Prepare by boiling 430 grm. of normal lead acetate, 130 grm. of litharge, and 1,000 c.c. of water for

half an hour. Allow the mixture to cool and settle and dilute the supernatant liquid to 1.25 sp. gr. with recently boiled water. Solid basic lead acetate may be substituted for the normal salt and litharge in the preparation of the solution.

(2) **Alumina Cream.**—Prepare a cold saturated solution of alum in water and divide into two unequal portions. Add a slight excess of ammonia to the larger portion and then add by degrees the remaining alum solution until a faintly acid reaction is secured.

Preparation of the Solution of Sugar for the Polarimeter.— The standard quantity of the sample is weighed out, introduced into a 100 c.c. flask and dissolved in about 50 c.c. of water. If this solution be clear and colourless it is diluted to 100 c.c. and introduced into the tube of the polarimeter. If the liquid is coloured to any notable extent, as is usually the case with commercial sugars, it has first to be decolourised. This clarification may be effected by means of either of the reagents noted above. It is advisable to reject the first runnings. The following method of clarification is very efficacious even under extremely unfavorable conditions: The normal quantity of sugar is dissolved in about 50 c.c. of water in a flask holding 100 c.c. According to the quality of the sample the solution will be (1) colourless but cloudy, (2) yellow, (3) brown, or (4) almost black. In the first case, add about 3 c.c. alumina cream and one drop of basic lead acetate solution. In the second case, the same volume of a lumina cream may be used, but the lead solution increased to 3 or 5 drops. In the third or fourth case add about 2 c.c. of a 10 % solution of sodium sulphite, and then the lead solution gradually, with constant shaking, till no further precipitate is produced. Whichever mode of clarification is adopted, the liquid is well agitated and allowed to stand at rest for a few minutes, to insure the complete separation of any precipitate. The flask is then filled nearly to the mark with water, and the froth allowed to rise to the surface, when it is destroyed by the cautious addition of a few drops of spirit or a single drop of ether. Water is then added exactly to the mark, the contents of the flask thoroughly mixed by agitation, and the liquid filtered through a dry filter.

The validity of the simple direct polarimetric reading of a standard solution of a commercial sugar as a measure of the actual amount of sucrose contained in it is the most vexed question[1] of industrial sugar

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[1] A difference of 0.2 or 0.3 per cent. on an importation of 5,000 or 10,000 tons obviously runs into large figures.

chemistry and the attention of chemists has been largely directed towards securing uniformity in the process. It is out of the question here to do more than briefly refer to some of the difficulties, especially as much that has been written on the matter is of a controversial nature.

Clarification with basic lead acetate is productive of error owing to the volume occupied by the precipitate. Further[1] excessive amounts exercise an appreciable effect on the optical activity and reducing power of any invert sugar contained in the sugar solution.[2]

The use of anhydrous basic lead acetate as proposed by Horne (*J. Amer. Chem. Soc.*, 1904, **26**, 186) enables these errors to be overcome. Excess is easily avoided since each particle of the powder added produces a precipitation and no more is added when the precipitation begins to be slight. In such cases, the filtrate is free from lead and clarification involves no appreciable error. This appears to be the most convenient and accurate method of clarifying sugar solutions.

In the case of low-grade products Watts and Tempany proceed as follows:

A double normal weight is dissolved in 100 c.c. partially clarified with dry basic lead acetate and the solution filtered. 50 c.c. of the dark coloured filtrate are saturated with sulphur dioxide and the lead precipitated as sulphite. The solution is diluted to 100 c.c. and filtered when a bright lemon-yellow filtrate is usually obtained.

Another mode of clarification is as follows: Solutions of alum or aluminum sulphate and of basic lead acetate are prepared of equivalent strengths, so that on mixing equal volumes and filtering no sulphate remains in solution. To the solution of sugar 5 c.c. of each of these liquids are added, the mixture shaken, made up to 100 c.c., and passed through a dry filter.

Some exceptionally dark cane sugars, and most beet-root molasses are not sufficiently decolourised by either of the above methods. In such cases a double normal quantity should be weighed out, and the solution clarified by sodium sulphite and basic-lead solution, as before described, a rather larger quantity of the latter liquid being employed. The solution is made up accurately to 100 c.c., filtered, and 50 c.c. of the filtrate treated with a saturated solution of sulphurous acid[3] until

[1] The question is fully discussed by Watts and Tempany, *J. Soc. Chem. Ind.*, 1907, **27**, 53.
[2] Formaldehyde, which is used in preserving saccharine solutions in sugar mills, if present in greater quantity than 1 c.c. of formalin per 100 c.c. of sugar solution, appreciably increases the rotation. See Norris, Bulletin 23, of Agriculture and Chemistry, Sandwich Islands, Feb. 19, 1908.
[3] Instead of employing a saturated solution of sulphurous acid it is convenient to bubble through the liquid a little sulphur dioxide.

the liquid smells strongly of the gas. About 2 grm. of purified animal charcoal[1] are then added, the liquid well shaken, made up exactly to 100 c.c., and filtered. By proceeding in this manner, a perfectly colourless or lemon-yellow solution may be obtained from the worst samples.[2]

Careful experiments as to the effect of basic lead acetate on the optical rotation of sucrose have been made by Bates and Blake (*J. Amer. Chem. Soc.*, 1907, **29,** 286) who find that a diminished polariscope reading is first caused and that further addition of the reagent produces a continuous rise in the rotation. This, they consider, is probably due to the formation of soluble lead saccharates having rotatory powers different from that of sucrose.

Pellet (*Bull. Soc. Chim. Sucr. Diot.*, 1906, **23,** 1466–1471) points out that in clarifying sugar solutions with basic lead acetate two sources of error are introduced: (1) The volume occupied by the precipitate causes the true concentration of the sugar solution to be greater than the apparent, thereby increasing the reading. (2) The precipitate retains a quantity of the sugar mechanically, thereby reducing the reading. Pellet's experiments show that these errors compensate each other and there is no necessity to make any correction.

To remove excess of lead from solutions clarified with lead acetate, anhydrous sodium carbonate or sodium sulphate is frequently used. A solution of double normal potassium oxalate (184.4 grm. in 1000 c.c.) is recommended by Sawyer (*J. Amer. Chem. Soc.*, 1904, **26,** 1631). One hundred c.c. are added to the clarified solution which is filtered after 15 minutes. The precipitate is granular and easily filtered and the oxalate does not interfere with the polarisation.

When basic lead acetate is used with solutions containing lævulose the lævulose lead compound must be destroyed by rendering the filtrate acid.

Basic lead acetate is not suitable for clarifying invert sugar products containing salts which yield insoluble compounds with lead as such precipitates carry down a certain amount of lævulose. Schrefeld (*2. Ver. dent. Zuckerind,* 1908, 947) recommends the use of *normal* lead acetate and obtains fairly accurate results in the case of molasses.

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[1]This is prepared by boiling 1 pound of freshly ground bone-charcoal in half a gallon of common yellow hydrochloric acid diluted with one gallon of water. The liquid is filtered through a linen bag, and the residue washed with hot water till free from acid, dried and ignited to full redness in a closed crucible. It is bottled while still warm, and kept carefully dry.

[2]See the articles on analysis of beet-root juice and molasses for precautions necessary for the removal of foreign optically active bodies from these substances. Watts and Tempany (*J. Soc. Chem. Ind.,* 1907, **27,** 53.) consider this method to be liable to serious errors by reason of the precipitate volumes.

To correct for the volume of a precipitate, Scheibler's method of dissolving normal weights of the sample in 100 and 200 c.c. may be used. The true reading is obtained by dividing the product of the two readings by their difference.

Solutions of beet sugar clarified electrically yield higher results on polarisation than when clarified by basic lead acetate, owing possibly to the presence of asparagine; this is obviated by the addition of acetic acid. F. G. Wiechmann (*Z. Ver. deutsch. Zukerind.*, 1906, 1056) recommends the following method of working:

26 grm. of sucrose are dissolved in 100 c.c. water at 20° and about 35 c.c. are introduced into each of the two compartments of an electrolytic cell, having a parchment diaphragm and lead electrodes. A current of 0.25 ampere is passed for five minutes, the anode liquid filtered, mixed with 10% of its volume of an 8% solution of acetic acid and polarised. The reading is increased by 10% to compensate for the dilution caused by the acetic acid.

Sucrose in presence of glucose in raw sugar, molasses, etc. *Clerget's Process.*

A solution of cane sugar which, before inversion, causes a deviation of 100 divisions to the right, after inversion has a lævorotatory power of 39 divisions at 10°. The change is consequently equivalent to a rotation of 139 divisions. Owing to the diminished optical power of lævulose at higher temperatures, this change is less the higher the temperature at which it is observed. At 0° the change by inversion equals 144 divisions; in general the value at $t°$ is given by the equation:

$$D = 144 - t/2.$$

By polarising a solution before and after inversion the change in the polarimetric reading due to the hydrolysis of the cane sugar present is found and if C be that part of the rotation produced by the uninverted liquid which is really due to the cane sugar contained in it

$$C = \frac{100\ D}{144 - t/2}$$

Adopting the International Commission methods when the polarisation before and after inversion is taken at 20°, and the normal weight is 26 grms.

$$C = \frac{100\ D}{132.66}$$

which at a temperature $t°$ C. becomes

$$C = \frac{100\ D.}{142.66 - t/2}$$

The (official in the United States) method of inversion (International Commission) is as follows: Take 50 c.c. of the normal sugar solution made up in the manner already described and freed from lead by treating with anhydrous sodium carbonate or sodium sulphate, place in a 100 c.c. flask, and add 25 c.c. of water. Then add, little by little, while rotating the flask, 5 c.c. of hydrochloric acid, containing 38.8 % of the acid (sp. gr. 1.188). Heat the flask after mixing in a water-bath which is at 70°. The temperature of the solution in the flask should reach 67° to 69° in two and one-half to three minutes. Maintain a temperature of as nearly 69° C. as possible during 7 to 7 and one-half minutes, making a total time of heating of ten minutes. Remove the flask and cool the contents rapidly to 20° and dilute to 100 c.c. Examine this solution in a tube provided with a lateral branch and a water jacket, passing a current of water around the tube to maintain a temperature of 20°.

The inversion may also be accomplished as follows: To 50 c.c. of the clarified solution, freed from lead, add 5 c.c. of a 38.8 % solution of hydrochloric acid and set aside during a period of 24 hours at a temperature not below 20°; or if the temperature be above 25° set aside for ten hours. Make up to 100 c.c. at 20° and polarise at that temperature. This reading must be multiplied by two, which gives the invert reading. In case it is necessary to work at a temperature other than 20° which is allowable within narrow limits, the volumes must be completed and both direct and invert polarisations must be made at exactly the same temperature.

Estimation of Cane Sugar in Presence of Raffinose.—The high dextrorotatory sugar, raffinose, often occurs in beet molasses and products. The Clerget method is only available when not more than one other optically active substance is present besides sucrose.

The normal weight (26 grm.) of raffinose anhydride in 100 c.c. water has a rotation of $+185.2°$ at 20° in a 200 mm. tube before and $+94.9°$ after inversion.[1]

$$S = \frac{0.5188\ P - I}{0.8454}$$

[1] Citric acid or a weak mineral acid should be used for inversion to avoid hydrolysing the melibiose section of raffinose.

where S = the amount of sucrose, P is the polarisation before and I that after inversion.

$$R \text{ (the amount of raffinose)} = \frac{P-S}{1.852} \quad .$$

When invert sugar is also present it is necessary to determine the cupric reducing power of the original and the inverted solution in addition to the above polarisations.

To detect raffinose in presence of sucrose, Neuberg and Marx (*Zeit. Ver. deut. Zuckerind.*, 1907, 453) make use of the formation of cupric reducing sugars by the action of emulsin on raffinose, this enzyme being without action on cane sugar. The presence of reducing sugars or of glucosides which are attacked by emulsin prevents the application of the test.

The Clerget method is applicable to the estimation of cane sugar only so long as other sugars, inulins, starches and glucosides, which are also inverted by acids, are not present. In such cases invertase may be used to effect hydrolysis.

Preparation of Invertase.—Invertase is a soluble enzyme present in yeast and very widely distributed in plants. It has the property of rapidly and completely effecting the transformation of cane sugar into invert sugar but is entirely without action on dextrose, lævulose, maltose or lactose. Indeed, the only other substances which are hydrolysed by invertase are trisaccharides like raffinose and sentianose which contain cane sugar in their molecule.

Invertase is most conveniently prepared as follows. Dry pressed yeast is crumbled as finely as possible and spread out in a thin layer on a sheet of porous paper in a dry, airy place; in the course of a day or two it dries to a light friable powder. When perfectly dry, it may be bottled and kept indefinitely. 5 grm. of this are shaken for an hour with 100 c.c. of water containing 0.5 c.c. of toluene and filtered bright. A few c.c. of this solution are added to the saccharine solution under examination, a little toluene is added and the mixture incubated in a corked vessel preferably at a raised temperature—37 to 50°—for a few hours. The determination of the optical rotatory activity and reducing power in the inverted solution is carried out in the ordinary manner, due allowance being made for the volume of enzyme solution added. This need only be 1 c.c. or less if the amount of sucrose to be inverted is small and at the higher

temperature the time required for complete inversion may be less than an hour.

Kjeldahl employed a little fresh washed yeast in presence of thymol to effect inversion, fermentation being prevented by the antiseptic.

The use of chloroform or ether as antiseptics is in general undesirable. The former must be got rid of by heating the liquid after inversion, as it exerts a cupric reducing action. Ether, unless very pure, may adversely affect the enzyme. Invertase may also be prepared by allowing brewer's yeast to liquefy—this takes a few days at 37° C. The filtered liquid has a high hydrolytic power. The enzyme may be partially purified by precipitation with alcohol and redissolution of the precipitate in a minimum of water. This extract keeps well in presence of toluene in closed vessels in the dark.

If a solution be inverted rapidly it is often advisable to add a minute trace of ammonia before polarising to obviate the error caused by mutarotation (see next paragraph).

Birotation.—Considerable confusion has in the past been introduced into optical saccharimetry owing to the changes in rotatory power shown by freshly dissolved sugars on keeping, a phenomenon known as "bi-rotation." This change has been shown by E. F. Armstrong and T. M. Lowry (*Trans. Chem. Soc.*, 1903, **83**, 1305, 1314) to be due to the mutual interconversion in solution of two isomerides of the sugar. The "birotation" of sugars is indeed a special case of a more general phenomenon to which Lowry has given the name "mutarotation," to show that its essential characteristic is a *change* of rotatory powers. (See *Trans. Chem. Soc.*, 1899, **75**, 212.) Most sugars exist in solution as a mixture of two forms in equilibrium. Thus in the case of glucose the anhydrous solid is the *a*-modification of high rotatory power which persists as such in the freshly made solution but slowly passes over in part into the β-form of low rotatory power. The change is much accelerated by impurities, particularly those of an alkaline nature. The addition of a trace of alkali to a freshly made solution of glucose causes a sudden fall in the rotation—this has been made use of to identify the various forms of this sugar.

All products, such as honeys, syrups, etc., which contain dextrose or other reducing sugars in the crystalline form or in supersaturated solution, exhibit the phenomenon of birotation. The constant rotation only should be employed in the Clerget formula, and to obtain this the solutions prepared for direct polarisation should be allowed to

stand over night before making the reading. In case it is desired to make the direct reading immediately the birotation may be destroyed by heating the neutral solution to boiling for a few minutes or by adding a few drops of strong ammonia before completing the volume.

Estimation of Sugars by Means of the Refractometer.—This method may be used in the same manner as the sp. gr. method, over which it has advantages in speed and ease of manipulation. The most recent experience shows that 'the refractometric method gives trustworthy results even with highly concentrated and very crude syrups. When using the Pulfrich instrument, as done by Stolle (*Zeit. Ver. deut. Zuckerind.*, 1901, 335, 469), 5 c.c. of solution are necessary; with the Abbé refractometer a few drops suffice. Working with the latter instrument, Tolman and Smith (*J. Amer. Chem. Soc.*, 1906, **28,** 1476) find that for the same concentration the index of refraction is practically the same for sucrose, maltose, lactose, dextrose, lævulose, and commercial glucose, but is somewhat higher for dextrin. The following table gives the refractive index for sucrose solutions of varying strengths at 20°. The temperature correction is practically the same as that for sp. gr.

Sucrose %	Index of refraction at 20°	Sucrose %	Index of refraction at 20°	Sucrose %	Index of refraction at 20°
1	1.3343	31	1.3828	61	1.4442
2	1.3357	32	1.3847	62	1.4465
3	1.3372	33	1.3865	63	1.4488
4	1.3387	34	1.3883	64	1.4511
5	1.3402	35	1.3902	65	1.4534
6	1.3417	36	1.3921	66	1.4557
7	1.3432	37	1.3940	67	1.4581
8	1.3447	38	1.3959	68	1.4605
9	1.3462	39	1.3978	69	1.4629
10	1.3477	40	1.3997	70	1.4653
11	1.3492	41	1.4017	71	1.4677
12	1.3508	42	1.4036	72	1.4701
13	1.3524	43	1.4056	73	1.4726
14	1.3539	44	1.4076	74	1.4751
15	1.3555	45	1.4096	75	1.4776
16	1.3572	46	1.4117	76	1.4801
17	1.3588	47	1.4137	77	1.4826
18	1.3604	48	1.4158	78	1.4851
19	1.3621	49	1.4179	79	1.4877
20	1.3637	50	1.4200	80	1.4903
21	1.3654	51	1.4221	81	1.4929
22	1.3671	52	1.4242	82	1.4955
23	1.3688	53	1.4263	83	1.4981
24	1.3705	54	1.4284	84	1.5007
25	1.3722	55	1.4306	85	1.5034
26	1.3739	56	1.4328	86	1.5061
27	1.3756	57	1.4351	87	1.5088
28	1.3774	58	1.4373	88	1.5115
29	1.3792	59	1.4396	89	1.5142
30	1.3810	60	1.4419	90	1.5170

Reactions of the Sugars as Reducing Agents.—Most of the carbohydrates, with the notable exception of cane sucrose and raffinose, possess marked activity as reducing agents.

In hot alkaline solution, the glucoses reduce picric acid to picramic acid, indigotin to indigo white, and change ferricyanides to ferrocyanides. Bismuth, mercury, silver, platinum, and gold salts are reduced to metal and ferric and cupric salts to ferrous and cuprous compounds respectively.

The reducing properties of sugars are best manifested and measured by their reaction on alkaline solutions of cupric and mercuric salts, and the processes in which these are employed require to be described in detail.

In the first place, the well-established standard processes will be dealt with, followed by a brief résumé of the more recent modifications of these methods, many of which perhaps still require confirmation by other workers before being universally adopted. They have been in many cases devised to solve the difficulties presented by special problems.

Reaction of Sugars with Cupric Salts in Alkaline Solution.— If a solution of cupric sulphate be mixed with a sufficient quantity of a saccharine liquid, no precipitate of copper hydroxide is produced on addition of sodium or potassium hydroxide. The liquid becomes deep blue, but remains clear. On raising the fluid to b. p. no visible change occurs if the liquid contained sucrose only, but, if any form of glucose is present, a yellow precipitate of cuprous hydroxide is produced, which quickly turns to cuprous oxide and becomes an orange-red. If the glucose is present in excess the blue of the solution entirely disappears. Instead of relying on a saccharine substance for the prevention of the precipitation of the cupric hydrate by the alkali it is far better to employ a tartrate, as in Fehling's solution.

The reducing action of certain forms of sugar on alkaline solutions of copper has been applied by different chemists in many ways, the precipitated cuprous oxide being weighed as such by several, by others converted into metallic copper or cupric oxide, and by others redissolved and estimated volumetrically. Some operators make the original process a volumetric one. The great majority of these modified processes are merely of historical interest and require no detailed description.

Fehling's Solution.

The alkaline solution of copper most commonly employed for the determination of sugars is that known as Fehling's which is essentially a solution of copper sodium tartrate containing a considerable quantity of sodium hydroxide. (For the nature of the salts existing in Fehling's solution see Masson & Steele, *Trans.*, 1899, **75**, 725, and Bullnheimer and Seitz, *Ber.*, 1900, **33**, 807.) It is best prepared in the following manner, known as Soxhlet's modification: 34.64 grm. weight of pure crystallised copper sulphate (free from iron and moisture) are dissolved in distilled water, and the solution diluted to 500 c.c. Seventy[1] grm. of sodium hydroxide of good quality (not less than 97 % NaOH) and 175 grm. of recrystallised potassium sodium tartrate are dissolved in about 400 c.c. of water and the solution diluted to 500 c.c. Fehling's solution is prepared by carefully adding the copper sulphate solution to an equal measure of the alkaline tartrate solution. It may be kept ready-mixed, but should in that case be carefully protected from air and light, as it is apt to undergo changes which render its indications unreliable. Before using it is desirable to ascertain its condition, by diluting a quantity with an equal volume of water and heating the liquid to boiling for a few minutes. It ought to remain perfectly clear. It is preferable to keep the copper and tartrate solutions separate, and mix them in equal measures at frequent intervals.

For the *detection of reducing sugar* in clear, colourless solution, all that is necessary is to neutralise any free acid and heat the liquid to boiling with a little Fehling's solution. If a yellow or orange-red turbidity or precipitate of cuprous oxide be produced, a reducing sugar, or some substance giving a similar reaction, is present. The glucoses, and maltose, reduce the copper solution with facility, but sucrose gives no reaction until after inversion.

If the liquid is much coloured it is difficult or impossible to recognise properly the reaction with Fehling's solution. Colouration of the liquid is still more objectionable if the sugar is to be estimated by the volumetric process. In such cases the sugar solution must be clarified by one of the methods employed for the preparation of a solution for the polarimeter (see page 309), but if lead has been employed it must be *completely* removed from the solution or the results of the test will be worthless.

Fehling's solution may be used volumetrically or gravimetrically.

[1]The A. O. A. C. uses 50 grm. of sodium hydroxide.

Both methods are capable of giving useful approximate results, but if any high degree of accuracy be sought it is essential that certain conditions of manipulation be strictly adhered to.

As a good approximate estimation of the amount of reducing sugar present in a liquid is often all that is requisite, it will be convenient to give methods by which such results can be readily obtained, and subsequently to describe the conditions which must be observed if a higher degree of accuracy be desired.

Volumetric Estimation of Reducing Sugars by Fehling's Solution.—The saccharine solution, prepared as already described and containing from 0.5 to 1.0 grm. of sugar per 100 c.c., is placed in a burette. Exactly 10 c.c. of the Fehling's solution are measured into a wide test-tube or small flask supported vertically by a clip. 30 c.c. of water are added, and a few fragments of tobacco-pipe stem dropped in to prevent bumping. The liquid is heated to boiling by applying a small flame, and the sugar solution run in, 2 c.c. at a time, boiling between each addition. When the blue colour of the liquid has nearly disappeared, the sugar solution should be added more cautiously, but it is desirable to effect the titration as rapidly as possible. The end of the reaction is reached when, on removing the flame and allowing the cuprous oxide to settle, the supernatant fluid appears colourless or faintly yellow when viewed against a white surface. If any doubt be felt as to the termination of the reaction, a few drops of the liquid may be filtered through a small filter into a mixture of acetic acid and dilute potassium ferrocyanide contained in a porcelain crucible or placed on a white plate. If copper be still present in the liquid, more or less brown colouration will be observed.

The results obtained by using Fehling's solution volumetrically are not generally so accurate as those of the gravimetric method. The operation should be *quickly* conducted.

The following are the weights of the principal kinds of sugar which, it is generally assumed, will reduce 10 c.c. of Fehling's solution prepared as described on page 318. Soxhlet's figures are given on page 321.

<blockquote>
10 c.c. Fehling solution = 0.0500 grm. of dextrose, lævulose, or invert sugar.
10 c.c. Fehling solution = 0.0475 grm. of cane sugar (after inversion).
10 c.c. Fehling solution = 0.0678 grm. of milk sugar (lactose).
10 c.c. Fehling solution = 0.0807 grm. of maltose.
</blockquote>

In all cases in which Fehling's solution is to be used volumetrically its true oxidising power under the conditions of the experiment should

be ascertained by actual trial. 0.0475 grm. of dry sucrose, after being inverted as described on page 313, and the solution neutralised, should exactly decolourise 10 c.c. of Fehling's solution.

The volumetric methods adopted by the A. O. A. C. are as follows:

(a) Applicable to Invert Sugar and Dextrose.

Place 10 c.c. of the mixed copper reagent in a large test-tube and add 10 c.c. of distilled water. Heat to boiling, and gradually add small portions of the solution of the material to be tested until the copper has been completely precipitated, boiling to complete the reaction after each addition. Two minutes' boiling is required for complete precipitation when the full amount of sugar solution has been added in one portion. When the end reaction is nearly reached and the amount of sugar solution to be added can no longer be judged by the colour of the solution, remove a small portion of the liquid and filter rapidly into a small porcelain crucible or on a test plate; acidify with dilute acetic acid, and test for copper with a dilute solution of potassium ferrocyanide. The sugar solution should be of such strength as will give a burette reading of 15 to 20 c.c. and the number of successive additions should be as small as possible.

Since the factor of calculation varies with the minute details of manipulation, every operator must determine a factor for himself, using a known solution of a pure sample of the sugar that he desires to determine, and keeping the conditions the same as those used for the determinations.

Standardise the solution for invert sugar in the following manner:

Dissolve 4.75 grm. of pure sucrose in 75 c.c. of water, add 5 c.c. of hydrochloric acid (sp. gr. 1.188), and invert as under the official method for sucrose, page 313. Neutralise the acid exactly with sodium hydroxide and dilute to 1 liter. 10 c.c. of this solution contains 0.050 grm. of invert sugar, which should reduce 10 c.c. of the copper solution; the copper solution should never be taken as a standard, but should be checked against the sugar. In case this method is used for determining dextrose, pure dextrose must be used in standardising the solution.

(b) Soxhlet's Method.

Make a preliminary titration to determine the approximate per centage of reducing sugar in the material under examination. Prepare

a solution which contains approximately 1% of reducing sugar. Place in a beaker 100 c.c. of the mixed copper reagent and approximately the amount of the sugar solution for its complete reduction. Boil for two minutes. Filter through a folded filter and test a portion of the filtrate for copper by use of acetic acid and potassium ferrocyanide. Repeat the test, changing the volume of sugar solution, until two successive amounts are found which differ by 0.1 c.c., one giving complete reduction and the other leaving a small amount of copper in solution. The mean of these two readings is taken as the volume of the solution required for the complete precipitation of 100 c.c. of the copper reagent.

Under these conditions 100 c.c. of the mixed copper reagent require 0.475 grm. of anhydrous dextrose or 0.494 grm. of invert sugar for complete reduction. Calculate the percentage by the following formula:

- V = the volume of the sugar solution required for the complete reduction of 100 c.c. of the copper reagent.

W = the weight of the sample in 1 c.c. of the sugar solution.

$$\text{Then} \quad \frac{100 \times 0.475}{VW} = \text{per cent. of dextrose,}$$

$$\text{or} \quad \frac{100 \times 0.494}{VW} = \text{per cent. of invert sugar.}$$

The titration of raw sugars, malt worts and other coloured commercial products with Fehling's solution, employing potassium ferrocyanide as indicator, is often anything but an accurate method and very tedious. When certain amino-compounds are present so much cuprous oxide may be dissolved that it is impossible to obtain an acidified filtrate which gives no colour with potassium ferrocyanide. Indicators, which respond to a minute trace of cupric salt and can be employed without filtering off a portion of the assay liquid, have been recently proposed. E. F. Harrison (*Pharm. Journ.*, 1903, **71**, 170) uses a solution of starch and potassium iodide which when acidified with acetic acid and brought into contact with a cupric salt liberates iodine and blue is developed.

Still more satisfactory is a solution of ferrous thiocyanate as suggested by A. R. Ling, T. Rendle and G. C. Jones (*Analyst*, 1905, **30**, 182; 1908, **33**, 160–170). When a drop of this on a white slab is brought into contact with a drop of a solution of a cupric salt the character-

istic red colour of ferric thiocyanate is produced. Ling's method
has been adopted by the Malt Analysis Committee of the Institute
of Brewing (*J. Inst. Brewing*, 1906, **12**, No. 1) and is given below:

Preparation of the Indicator.—1 grm. of ferrous ammonium
sulphate and 1.5 grm. of ammonium thiocyanate are dissolved in 10 c.c.
of water at a moderate temperature, say at 120° F., and immediately
cooled; 2.5 c.c. of concentrated hydrochloric acid are then added.
The solution so obtained is invariably brownish-red, due to the presence
of ferric salt, which latter must be reduced. For this purpose, zinc
dust is the most satisfactory reagent, and a mere trace is sufficient to
decolourise the solution if pure reagents have been employed.

When kept for some hours, the indicator redevelops the red by oxi-
dation. It may, however, be decolourised by the addition of a further
quantity of zinc dust, but its delicacy is decreased after it has been
decolourised several times. For practical purposes the indicator
may be too delicate and it is recommended to prepare it the day
before it is required for use, as it gives the best results after the second
decolourisation.

The method of titration is as follows: Freshly mixed Fehling's solu-
tion (10 c.c.) is accurately measured into a 200 c.c. boiling flask and
raised to boiling. The sugar solution, which should be adjusted to such
a strength that 20 to 30 c.c. of it are required to reduce 10 c.c. of Feh-
ling's solution, is then run into the boiling liquid in small amounts,
commencing with 5 c.c. After each addition of sugar solution, the mix-
ture is boiled, the liquid being kept rotated. About a dozen drops of
the indicator are placed on a porcelain or opal glass slab and when it
is judged that the precipitation of cuprous oxide is complete, a drop of
the liquid is withdrawn by a clear glass rod or by a capillary tube and
brought in contact with the middle of a drop of the indicator on the
slab. The test must be carried out rapidly. It is also essential to
perform the titration as rapidly as possible, as an atmosphere of steam
is then kept in the neck of the flask and the influence of atmospheric
oxygen is avoided. At the final point the liquid is boiled for about
two seconds. As in the ordinary volumetric method, the first titration
may only give approximate results and a second or third will then be
necessary to establish the end-point accurately. However, when the
operator has gained experience the first titration is as much to be relied
on as succeeding ones. One titration takes about 3 minutes. The
authors claim the average error of the method to be about 1 in 300.

Gravimetric Estimation of Reducing Sugars by Fehling's Solution.—This method gives very accurate results provided the detal is of manipulation are closely attended to.

Allihn's Method.—34.639 grm. of crystallized copper sulphate are dissolved in water and diluted to 500 c.c. 173 grm. of sodium potassium tartrate and 125 grm. of potassium hydroxide are likewise dissolved in water and diluted to 700 c.c. A solution of the material to be examined is prepared so as not to contain more than 1 % of dextrose. 30 c.c. of each of the reagent solutions and 60 c.c. of water are placed in a beaker and heated to boiling; 25 c.c. of the dextrose solution are added and the boiling continued for exactly two minutes. The liquid is filtered immediately without dilution and the amount of copper contained in the cuprous oxide determined by one of the following methods:

(1) Reduction in Hydrogen.

Filter the cuprous oxide immediately through a weighed filtering tube made of hard glass, using suction. Support the asbestos film in the filtering tube with a perforated disk or cone of platinum and wash free from loose fibres before weighing; moisten previous to the filtration. Provide the tube with a detachable funnel during the filtration, so that none of the precipitate accumulates near the top, where it could be removed by the cork used during the reduction of the cuprous oxide. Transfer all the precipitate to the filter and thoroughly wash with hot water, following the water by alcohol and ether successively. After being dried, connect the tube with an apparatus for supplying a continuous current of dry hydrogen, gently heat until the cuprous oxide is completely reduced to the metallic state, cool in the current of hydrogen, and weigh. If preferred, a gooch crucible may be used for the filtration.

(2) Electrolytic Deposition from Sulphuric Acid Solution.

Filter the cuprous oxide in a gooch, wash the beaker and precipitate thoroughly with hot water without any effort to transfer the precipitate to the filter. Wash the asbestos film and the adhering cuprous oxide into the beaker by means of hot dilute nitric acid. After the copper is all in solution, refilter through a gooch with a thin film of asbestos and wash thoroughly with hot water. Add 10 c.c. of dilute sulphuric

acid, containing 200 c.c. of sulphuric acid (sp. gr. 1.84) in 1000 c.c., and evaporate the filtrate on the steam-bath until the copper salt has largely crystallized. Heat carefully on a hot plate or over a piece of asbestos board until the evolution of white fumes shows that the excess of nitric acid is removed. Add from 8 to 10 drops of nitric acid (sp. gr. 1.42) and rinse into a platinum dish of from 100 to 125 c.c. capacity. Precipitate the copper by electrolysis. Wash thoroughly with water before breaking the current, remove the dish from the circuit, wash with alcohol and ether successively, dry at about 50° and weigh. If preferred, the electrolysis can be conducted in a beaker, the copper being deposited upon a weighed platinum cylinder.

(3) Electrolytic Deposition from Sulphuric and Nitric Acid Solution.

Filter and wash as under (2). Transfer the asbestos film from the crucible to the beaker by means of a glass rod and rinse the crucible with about 30 c.c. of a boiling mixture of dilute sulphuric and nitric acids, containing 65 c.c. of sulphuric acid (sp. gr. 1.84) and 50 c.c. of nitric acid (sp. gr. 1.42) in 1000 c.c. Heat and agitate until solution is complete; filter and electrolyse as under (2).

(4) Electrolytic Deposition from Nitric Acid Solution.

Filter and wash as under (2). Transfer the asbestos film and adhering oxide to the beaker. Dissolve the oxide still remaining in the crucible by means of 2 c.c. of nitric acid (sp. gr. 1.42), adding it with a pipette and receiving the solution in the beaker containing the asbestos film. Rinse the crucible with a jet of water, allow the rinsings to flow into the beaker. Heat the contents of the beaker until the copper is all in solution, filter, dilute the filtrate to a volume of 100 c.c. or more, and electrolyse. When a nitrate solution is electrolysed, the first washing of the deposit should be made with water acidified with sulphuric acid, in order that the nitric acid may be all removed before the current is interrupted.

(5) Volumetric Permanganate Method.

Filter and wash the cuprous oxide as described for method (2). Transfer the asbestos film to the beaker, add about 30 c.c. of hot water, and heat the precipitate and asbestos thoroughly. Rinse the crucible

with 50 c.c. of a hot saturated solution of ferric sulphate in 20 % sulphuric acid, receiving the rinsings in the beaker containing the precipitate. After the cuprous oxide is dissolved, wash the solution into a large Erlenmeyer flask and immediately titrate with a standard solution of potassium permanganate. 1 c.c. of the permanganate solution should equal 0.010 grm. of copper. In order to determine the strength of this solution, make 6 or more determinations with the same sugar solution, titrating one-half of the precipitate obtained, and determining the copper in the others by electrolysis. The average weight of copper obtained by electrolysis, divided by the average number of cubic centimetres of permanganate solution required for the titration is equal to the weight of copper equivalent to 1 c.c. of the standard permanganate solution. A solution standardised with iron or oxalic acid will give too low results.

(6) Direct Weighing of Cuprous Oxide.

Prepare a gooch with an asbestos felt. First thoroughly wash the asbestos with water to remove small particles, then follow successively with 10 c.c. of alcohol and 10 c.c. of ether, and dry the crucible and contents thirty minutes in a water-oven at the temperature of boiling water.

Collect the precipitated cuprous oxide on the felt as usual, thoroughly wash with hot water, then with 10 c.c. of alcohol, and finally with 10 c.c. of ether. Dry the precipitate 30 minutes in a water-oven at the temperature of boiling water; cool and weigh. The weight of cuprous oxide multiplied by 0.8883 gives the weight of metallic copper.

The weight of dextrose corresponding to the copper obtained is found by the following table:

ALLIHN'S TABLE FOR THE ESTIMATION OF DEXTROSE.

Milligrams of copper	Milligrams of dextrose	Milligrams of copper	Milligrams of dextrose	Milligrams of copper	Milligrams of dextrose	Milligrams of copper	Milligrams of dextrose	Milligrams of copper	Milligrams of dextrose
10	6.1	20	11.0	30	16.0	40	20.9	50	25.9
11	6.6	21	11.5	31	16.5	41	21.4	51	26.4
12	7.1	22	12.0	32	17.0	42	21.9	52	26.9
13	7.6	23	12.5	33	17.5	43	22.4	53	27.4
14	8.1	24	13.0	34	18.0	44	22.9	54	27.9
15	8.6	25	13.5	35	18.5	45	23.4	55	28.4
16	9.0	26	14.0	36	18.9	46	23.9	56	28.8
17	9.5	27	14.5	37	19.4	47	24.4	57	29.3
18	10.0	28	15.0	38	19.9	48	24.9	58	29.8
19	10.5	29	15.5	39	20.4	49	25.4	59	30.3

ALLIHN'S TABLE FOR THE ESTIMATION OF DEXTROSE.— CONTINUED.

Milligrams of copper	Milligrams of dextrose	Milligrams of copper	Milligrams of dextrose	Milligrams of copper	Milligrams of dextrose	Milligrams of copper	Milligrams of dextrose	Milligrams of copper	Milligrams of dextrose
60	30.8	126	64.2	192	98.4	258	133.5	324	169.7
61	31.3	127	64.7	193	98.9	259	134.1	325	170.3
62	31.8	128	65.2	194	99.4	260	134.6	326	170.9
63	32.3	129	65.7	195	100.0	261	135.1	327	171.4
64	32.8	130	66.2	196	100.5	262	135.7	328	172.0
65	33.3	131	66.7	197	101.0	263	136.2	329	172.5
66	33.8	132	67.2	198	101.5	264	136.8	330	173.1
67	34.3	133	67.7	199	102.0	265	137.3	331	173.7
68	34.8	134	68.2	200	102.6	266	137.8	332	174.2
69	35.3	135	68.8	201	103.1	267	138.4	333	174.8
70	35.8	136	69.3	202	103.7	268	138.9	334	175.3
71	36.3	137	69.8	203	104.2	269	139.5	335	175.9
72	36.8	138	70.3	204	104.7	270	140.0	336	176.5
73	37.3	139	70.8	205	105.3	271	140.6	337	177.0
74	37.8	140	71.3	206	105.8	272	141.1	338	177.6
75	38.3	141	71.8	207	106.3	273	141.7	339	178.1
76	38.8	142	72.3	208	106.8	274	142.2	340	178.7
77	39.3	143	72.9	209	107.4	275	142.8	341	179.3
78	39.8	144	73.4	210	107.9	276	143.3	342	179.8
79	40.3	145	73.9	211	108.4	277	143.9	343	180.4
80	40.8	146	74.4	212	109.0	278	144.4	344	180.9
81	41.3	147	74.9	213	109.5	279	145.0	345	181.5
82	41.8	148	75.5	214	110.0	280	145.5	346	182.1
83	42.3	149	76.0	215	110.6	281	146.1	347	182.6
84	42.8	150	76.5	216	111.1	282	146.6	348	183.2
85	43.4	151	77.0	217	111.6	283	147.2	349	183.7
86	43.9	152	77.5	218	112.1	284	147.7	350	184.3
87	44.4	153	78.1	219	112.7	285	148.3	351	184.9
88	44.9	154	78.6	220	113.2	286	148.8	352	185.4
89	45.4	155	79.1	221	113.7	287	149.4	353	186.0
90	45.9	156	79.6	222	114.3	288	149.9	354	186.6
91	46.4	157	80.1	223	114.8	289	150.5	355	187.2
92	46.9	158	80.7	224	115.3	290	151.0	356	187.7
93	47.4	159	81.2	225	115.9	291	151.6	357	188.3
94	47.9	160	81.7	226	116.4	292	152.1	358	188.9
95	48.4	161	82.2	227	116.9	293	152.7	359	189.4
96	48.9	162	82.7	228	117.4	294	153.2	360	190.0
97	49.4	163	83.3	229	118.0	295	153.8	361	190.6
98	49.9	164	83.8	230	118.5	296	154.3	362	191.1
99	50.4	165	84.3	231	119.0	297	154.9	363	191.7
100	50.9	166	84.8	232	119.6	298	155.4	364	192.3
101	51.4	167	85.3	233	120.1	299	156.0	365	192.9
102	51.9	168	85.9	234	120.7	300	156.5	366	193.4
103	52.4	169	86.4	235	121.2	301	157.1	367	194.0
104	52.9	170	86.9	236	121.7	302	157.6	368	194.6
105	53.5	171	87.4	237	122.3	303	158.2	369	195.1
106	54.0	172	87.9	238	122.8	304	158.7	370	195.7
107	54.5	173	88.5	239	123.4	305	159.3	371	196.3
108	55.0	174	89.0	240	123.9	306	159.8	372	196.8
109	55.5	175	89.5	241	124.4	307	160.4	373	197.4
110	56.0	176	90.0	242	125.0	308	160.9	374	198.0
111	56.5	177	90.5	243	125.5	309	161.5	375	198.6
112	57.0	178	91.1	244	126.0	310	162.0	376	199.1
113	57.5	179	91.6	245	126.6	311	162.6	377	199.7
114	58.0	180	92.1	246	127.1	312	163.1	378	200.3
115	58.6	181	92.6	247	127.6	313	163.7	379	200.8
116	59.1	182	93.1	248	128.1	314	164.2	380	201.4
117	59.6	183	93.7	249	128.7	315	164.8	381	202.0
118	60.1	184	94.2	250	129.2	316	165.3	382	202.5
119	60.6	185	94.7	251	129.7	317	165.9	383	203.1
120	61.1	186	95.2	252	130.3	318	166.4	384	203.7
121	61.6	187	95.7	253	130.8	319	167.0	385	204.3
122	62.1	188	96.3	254	131.4	320	167.5	386	204.8
123	62.6	189	96.8	255	131.9	321	168.1	387	205.4
124	63.1	190	97.3	256	132.4	322	168.6	388	206.0
125	63.7	191	97.8	257	133.0	323	169.2	389	206.5

ALLIHNS' TABLE FOR THE ESTIMATION OF DEXTROSE.—
CONTINUED.

Milli-grams of copper	Milli-grams of dextrose	Milli-grams of copper	Milli-grams of dextrose	Milli-grams of copper	Milli-grams of dextrose	Milli-grams of copper	Milli-grams of dextrose	Milli-grams of copper	Milli-grams of dextrose
390	207.1	405	215.8	420	224.5	435	233.4	450	242.2
391	207.7	406	216.4	421	225.1	436	233.9	451	242.8
392	208.3	407	217.0	422	225.7	437	234.5	452	243.4
393	208.8	408	217.5	423	226.3	438	235.1	453	244.0
394	209.4	409	218.1	424	229.9	439	235.7	454	244.6
395	210.0	410	218.7	225	227.5	440	236.3	455	245.2
396	210.6	411	219.3	426	228.0	441	236.9	456	245.7
397	211.2	412	219.9	427	228.6	442	237.5	457	246.3
398	211.7	413	220.4	428	229.2	443	238.1	458	246.9
399	212.3	414	221.0	429	229.8	444	238.7	459	247.5
400	212.9	415	221.6	430	230.4	445	239.3	460	248.1
401	213.5	416	222.2	431	231.0	446	239.8	461	248.7
402	214.1	417	222.8	432	231.6	447	240.4	462	249.3
403	214.6	418	223.3	433	232.2	448	241.0	463	249.9
404	215.2	419	223.9	434	232.8	449	241.6		

An alternative method which is often employed is as follows: 10 or 20 c.c. of each Fehling solution are placed in a beaker and diluted with 50 c.c. of boiling, well boiled, water. The beaker is then immersed in boiling water bath for 6 minutes, at the end of which time (the liquid being still perfectly clear) a known volume of the solution of the reducing sugar is added and the beaker kept in the boiling water for a further 12 minutes. It is advisable to dilute the sugar solution to contain about 0.5% reducing sugar. The solution should show blue at the end of the heating; if not, the assay should be repeated, using less saccharine liquid. After 12 minutes' heating the precipitated cuprous oxide is rapidly filtered, washed with boiling, well boiled, water, dried and ignited in porcelain. It is convenient to use a porcelain gooch crucible and asbestos wool for filtering. The red precipitate may be ignited to black cupric oxide in this. It is cooled under a desiccator and weighed as rapidly as possible as it is extremely hygroscopic.

The red precipitate may also be washed with water, alcohol and ether and weighed direct or it may be filtered in a hard glass tube and reduced in hydrogen or it may be redissolved and determined by electrolysis. (See methods 1 to 6 above.)

The following factors may be employed for calculating the weight of copper or copper oxide obtained to the corresponding quantities of the principal kinds of sugar:

	Glucose $C_6H_{12}O_6$	Cane sugar $C_{12}H_{22}O_{11}$ (after inversion)	Milk sugar $C_{12}H_{22}O_{11}$	Maltose $C_{12}H_{22}O_{11}$
Copper	.5634	.5395	.7707	.9089
Cuprous oxide	.5042	.4790	.6843	.8132
Cupric oxide	.4535	.4308	.6153	.7314

Thus, if a solution of o.1 grm. of a sample of sucrose has been inverted and precipitated as above described, and the resultant cupric oxide weighs 0.198 grm., then the total quantity of sugar (expressed as sucrose) is—

$$o.198 \times .4308 = .085298 = 85.3\%.$$

For the determination of small quantities of reducing sugar in materials containing a high percentage of sucrose slightly modified methods are advisable. Those adopted by the A. O. A. C. are as follows:

(a) Estimation in materials containing 1% or less of invert sugar and 99% or more of sucrose:

Prepare the solution of the material to be examined so as to contain 20 grm. in 100 c.c. free from suspended impurities by filtration and from soluble impurities by basic lead acetate, removing the excess of lead of means by sodium carbonate. Place 50 c.c. of the mixed copper reagent and 50 c.c. of the sugar solution in a beaker of 250 c.c. capacity. Heat this mixture at such a rate that approximately four minutes are required to bring it to boiling, and boil for exactly 2 minutes. Add 100 c.c. of cold, recently boiled, distilled water. Filter immediately through asbestos and estimate the copper by one of the methods given on pages 323 to 325.

Obtain the corresponding percentage of invert sugar by the use of the following table:

Hertzfeld's table for the estimation of invert sugar in materials containing 1%
or less of invert sugar and 99 % or more of sucrose.

Copper reduced by 10 grms. of material	Invert sugar	Copper reduced by 10 grms. of material	Invert sugar	Copper reduced by 10 grms. of material	Invert sugar
Milligrams	Per cent.	Milligrams	Per cent.	Milligrams	Per cent.
50	0.05	120	0.40	190	0.79
55	0.07	125	0.43	195	0.82
60	0.09	130	0.45	200	0.85
65	0.11	135	0.48	205	0.88
70	0.14	140	0.51	210	0.90
75	0.16	145	0.53	215	0.93
80	0.19	150	0.56	220	0.96
85	0.21	155	0.59	225	0.99
90	0.24	160	0.62	230	1.02
95	0.27	165	0.65	235	1.05
100	0.30	170	0.68	240	1.07
105	0.32	175	0.71	245	1.10
110	0.35	180	0.74		
115	0.38	185	0.76		

(b) Method for Materials Containing More than 1 per cent. of Invert Sugar.

—Prepare a solution of the material to be examined in such a manner that it contains 20 grm. in 100 c.c. after clarification and the removal of the excess of lead. Prepare a series of solutions in large test-tubes by adding 1, 2, 3, 4, 5, etc., c.c. of this solution to each successively. Add 5 c.c. of the mixed copper reagent to each, heat to boiling, boil two minutes, and filter. Note the volume of sugar solution which gives the filtrate lightest in tint, but still distinctly blue. Place 20 times this volume of the sugar solution in a 100 c.c. flask, dilute to the mark, and mix well. Use 50 c.c. of the solution for the determination, which is conducted as described under (a). For the calculation of the result use the following formulas and table of factors of Meissl and Hiller:

Let Cu = the weight of copper obtained;

   P = the polarisation of the sample;

   W = the weight of the sample in the 50 c.c. of the solution used for determination;

   F = the factor obtained from the table for conversion of copper to invert sugar;

   $\dfrac{Cu}{.2}$ = approximate absolute weight of invert sugar = Z;

$$Z \times \frac{100}{W} = \text{approximate per cent. of invert sugar} = y;$$

$$\frac{100\ P}{P+y} = R, \text{ relative number for sucrose;}$$

$100 - R = I$, relative number for invert sugar;

$$\frac{Cu\ F}{W} = \text{per cent. of invert sugar.}$$

Z facilitates reading the vertical columns; and the ratio of R to I, the horizontal columns of the table, for the purpose of finding the factor (F) for calculation of copper to invert sugar.

Example.—The polarisation of a sugar is 86.4, and 3.256 grm. of it (W) are equivalent to 0.290 grm. of copper. Then:

$$\frac{Cu}{2} = \frac{.290}{2} = 0.145 = Z$$

$$Z \times \frac{100}{W} = 0.145 \times \frac{100}{3.256} = 4.45 = Y$$

$$\frac{100\ P}{P+y} = \frac{8640}{86.4+4.45} = 95.1 = R$$

$$100 - R = 100 - 95.1 = I = 4.9$$
$$R : I = 95.1 : 4.9$$

By consulting the table it will be seen that the vertical column headed 150 is nearest to Z, 145, and the horizontal column headed 95:5 is nearest to the ratio of R to I, 95.1:4.9. Where these columns meet we find the factor 51.2, which enters into the final calculation:

$$\frac{Cu\ F}{W} = \frac{0.290 \times 51.2}{3.256} = 4.56 \text{ per cent. of invert sugar.}$$

Meissl and Hiller's factors for estimations in materials in which, of the total sugars present, 1% or more is invert sugar, and 99% or less is sucrose.

Ratio of sucrose to invert sugar-R:I.	Approximate absolute weight of invert sugar. = Z						
	200 milligrms.	175 milligrms.	150 milligrms.	125 milligrms.	100 milligrms.	75 milligrms.	50 milligrms.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
0:100	56.4	55.4	54.5	53.8	53.2	53.0	53.0
10:90	56.3	55.3	54.4	53.8	53.2	52.9	52.9
20:80	56.2	55.2	54.3	53.7	53.2	52.7	52.7
30:70	56.1	55.1	54.2	53.7	53.2	52.6	52.6
40:60	55.9	55.0	54.1	53.6	53.1	52.5	52.4
50:50	55.7	54.9	54.0	53.5	53.1	52.3	52.2
60:40	55.6	54.7	53.8	53.2	52.8	52.1	51.9
70:30	55.5	54.5	53.5	52.9	52.5	51.9	51.6
80:20	55.4	54.3	53.3	52.7	52.2	51.7	51.3
90:10	54.6	53.6	53.1	52.6	52.1	51.6	51.2
91:9	54.1	53.6	52.6	52.1	51.6	51.2	50.7
92:8	53.6	53.1	52.1	51.6	51.2	50.7	50.3
93:7	53.6	53.1	52.1	51.2	50.7	50.3	49.8
94:6	53.1	52.6	51.6	50.7	50.3	49.8	48.9
95:5	52.6	52.1	51.2	50.3	49.4	48.9	48.5
96:4	52.1	51.2	50.7	49.8	48.9	47.7	46.9
97:3	50.7	50.3	49.8	48.9	47.7	46.2	45.1
98:2	49.9	48.9	48.5	47.3	45.8	43.3	40.0
99:1	47.7	47.3	46.5	45.1	43.3	41.2	38.1

Titration by Pavy's Ammoniacal Cupric Solution.—This modification of the ordinary mode of using Fehling's solution for the estimation of reducing sugars is based on the fact that in presence of a sufficient excess of ammonium hydroxide the cuprous oxide is not precipitated, but forms a *colourless* solution, so that the end of the reaction is indicated by the decolourisation of the blue liquid. As the ammoniacal cuprous solution is extremely oxidisable, the blue colour being restored by oxidation, it is necessary to avoid access of air. This is best done by attaching the nose of the Mohr's burette containing the sugar solution to a tube passing through the india-rubber stopper of a flask containing the copper solution. A second tube conveys the steam and ammoniacal gas into a flask of cold water. It is desirable to allow the end of the tube to dip into a little mercury placed at the bottom of the water, so as to prevent any tendency to "suck back." A still better arrangement is to pass (by a third tube) a slow current of hydrogen or illuminating gas through the flask containing the boiling copper solution.

To prepare the ammoniacal solution, 120 c.c. of the ordinary Fehling's solution (see page 318) should be mixed with 300 c.c. of strong aqueous ammonia (sp. gr. 0.880), and with 400 c.c. of sodium hydroxide solution of 1.14 sp. gr. (=12 %). The mixture is then made up to 1000 c.c. One hundred c.c. % of this solution has the same oxidising

power on glucose as 10 c.c. of the ordinary Fehling's solution, that is, it corresponds to .050 grm.

In carrying out the process, 100 c.c. of the above solution are placed in the flask, a few fragments of pumice or tobacco-pipe added, the tubes and burette adjusted, and the liquid brought to ebullition. The sugar solution is then gradually run in from the burette, the boiling being continued regularly. The process is at an end when the blue colour of the liquid is wholly destroyed. The end-reaction is very sharply marked, but the reduction occurs more slowly than with the ordinary Fehling's solution. The process is often a very useful one, especially for the rapid assay of impure sacchar ne liquids, such as beer-worts.

O. Hehner has shown (*Analyst*, 1881, **6,** 218) that the presence of alkaline tartrates and carbonates, gravely affects the accuracy of indications obtained by Pavy's solution.

Pavy's solution has been very largely used in clinical chemistry, in particular in urine analysis. It has, however, many practical disadvantages, *e.g.*, the inconvenience of working with an ammoniacal solution and the great dilution as compared with the ordinary Fehling solution. It is, in fact, only applicable for solution containing from 1 to 10% the errors involved being too great in the case of larger amounts. Its application in chemistry, though giving very trustworthy results in experienced hands, should be practiced with caution.

Pavy's solution is of the greatest service in such cases as studied by Croft Hill (*Trans. Chem. Soc.*, 1898, **73,** 634) and E. F. Armstrong, in which it was required to ascertain very accurately clinical proportions of maltose and glucose in solutions the total sugar content of which remained constant.

Blythe ascertains the end reaction more accurately by bubbling air through the liquid directly the decolourisation is complete. The blue colour should reappear after a very few seconds unless too much sugar solution has been added when a longer time elapses.

Pavy's solution possesses a different oxidising power on maltose and lactose from that exerted by Fehling's test. Its reaction on invert sugar is, under the above-described conditions, only five-sixths of that exerted by Fehling's solution. Hence 120 c.c. of the latter are employed in making the ammoniacal solution, instead of 100, as would be the case if they were strictly equivalent.

Barfoed's reagent is prepared by dissolving 13.3 grm. of crystallised neutral copper acetate in 200 c.c. of 1 % acetic acid. (*Zeit. Anal. Chem.*, 1873, 12, 27) It forms a delicate test for dextrose and, moreover, is not reduced by either maltose or lactose under certain conditions—*i. e.*, less than two minutes' heating. More prolonged heating will cause hydrolysis of the disaccharides and reduction. The method has been applied quantitatively, but its indications are unreliable.

The behaviour of the reagent with dextrose, maltose, lactose and sucrose has quite recently been investigated by Hinkel and Sherman (*J. Amer. Chem. Soc.*, 1907, 29, 1744) who claim that using 5 c.c. of the reagent the presence of 0.0004 grm. dextrose can be shown, either alone or mixed with di-saccharides provided that the total weight of disaccharide does not exceed 0.02 grm. The test is best performed by heating in test-tubes in a boiling water-bath for 3 minutes. Under these conditions lactose or maltose do not cause reduction until heated for 9 or 10 minutes. Roaf (*Bio. Chem. Journ.*, 1908, 3, 182) has made use of the method to demonstrate the hydrolysis of lactose and maltose by enzymes.

In addition to the standard methods just described, numerous other copper solutions have been recommended and many suggestions made as to the unification of methods.

Preference is sometimes given to *Violette's* solution, prepared by dissolving (1) 34.66 grm. of crystallised copper sulphate and making up to 500 c.c.; and (2) 200 grm. of Rochelle salt and 130 grm. of sodium hydroxide and making up to 500 c.c. Equal volumes of the 2 solutions are mixed as required; 100 c.c. of the mixture are equal to 0.5 grm. of invert sugar.

H. Pellet (*Zeit. Ver. deut. Zuckerind.*, 1906, 1012) using Violette's solution, modifies the gravimetric process as follows: 40 c.c. of the copper solution and 10 to 20 c.c. of the sugar solution, which must not contain more than 5 to 10 grm. of reducing sugar per 1000 c.c., are mixed in a Jena glass flask, the volume being made up to 60 c.c. and heated in a boiling water-bath which contains so much water that the surface of the outer liquid is slightly above that of the solution in the flask. The solution is heated to from 85 to 88,° and kept at this temperature for 3 minutes.

50 c.c. of distilled water are then added, the flask is shaken, the cuprous oxide allowed to settle somewhat, and then filtered off

through an ashless filter (9 to 11 cm. diameter) previously moistened with water and finally washed with hot water until the washings are neutral. This precipitate and filtrate are incinerated (preferably in a muffle heated with gas or electricity), the cupric oxide being weighed. From 2 to 5 mg. must be subtracted from the weight of cupric oxide to compensate for the absorption of salts by the filter-paper; the actual amount must be directly determined. The amount of invert sugar is determined by multiplying the weight of cupric oxide by 0.454. For very accurate work, however, the coefficient must be determined by a control analysis with invert sugar. The above method possesses the following advantages over those which involve boiling the copper and sugar solutions: (1) several determinations can be made simultaneously; (2) heating is very uniform; (3) reduction is complete; (4) influence of secondary products, and especially of sucrose on the copper solution, is diminished.

· The method gives very concordant results, and clarification with lead acetate is unnecessary.

. Wiechmann (Zeit. Ver. deut. Zuckerind., 1907, 65) proposes on behalf of the International Commission the adoption of the following procedure for the examination of liquid sugar products. Soxhlet's modification of Fehling's solution is to be used.

26 grm. of the syrup are dissolved in water in a 100 c.c. flask, clarified with basic lead acetate, excess of lead being precipitated by 10% sodium chloride or sulphate; the solution is made up to 100 c.c., shaken and filtered.

1. **Total Sugar.**—50 c.c. of this solution are inverted by 5 minutes' heating at 67 to 70° with 5 c.c. of hydrochloric acid (sp. gr. 1.188). 50 c.c. of this are diluted to 1000 c.c. and 25 c.c. of this last solution, corresponding to 0.1625 grm. of syrup, are neutralised with 25 c.c. of dilute sodium carbonate (1.7 grm. per 1000 c.c.). 50 c.c. of Fehling's solution are added, the mixture heated to boiling in about 4 minutes, kept boiling for 3 minutes and the cuprous oxide filtered on an asbestos filter and weighed as such and multiplied by 0.888 to give the equivalent amount of copper.

2. **Reducing Sugar.**—4 c.c. of the clarified solution are made up to 100 c.c., 50 c.c. of this are boiled with 50 c.c. of Fehling's solution as above described. The difference in the two estimations represents the real amount of sucrose present. The original paper contains very complete instructions throughout.

An almost similar procedure is recommended by Munson and Walker (*J. Amer. Chem. Soc.*, 1906, **28**, 663) who have drawn up tables for use with the method. The preparation of the asbestos for filtering consists of a prolonged digestion with hydrochloric acid, followed by digestion with sodium hydroxide solution, extraction with boiling alkaline tartrate solution and digestion with nitric acid.

To avoid the injurious action of alkali hydroxides, Benedict (*J. Bio. Chem.*, 1907, **3**, 101) recommends the following solutions:

a. 69.3 grm. crystallized copper sulphate to 1000 c.c.

b. 346 grm. sodium potassium tartrate and 200 c.c. anhydrous sodium cabonate to 1000 c.c.

c 200 grm. of potassium thiocyanate. Equal volumes of these are mixed in the order indicated, more sodium carbonate added, the liquid boiled and the sugar solution run in till no further precipitate of cuprous thiocyanate is formed and the liquid is perfectly decolourised (see page 322).

Bang's method (*Biochem. Zeits.*, 1906, **2**, 271) is based on the fact that cuprous oxide in presence of potassium thiocyanate forms cuprous thiocyanate if the solution contains only alkali carbonate and no alkali hydroxide. The excess of copper, not reduced by the sugar, is determined by conversion into cuprous thiocyanate by hydroxylamine. Two hundred and fifty grm. of potassium carbonate, 50 grm. of potassium hydrogen carbonate and 200 grm. of potassium thiocyanate are dissolved in about 600 c.c. of water at 50–60°, cooled to 30°, and 12.5 grm. of copper sulphate dissolved in 75 c.c. of water added. After standing for 24 hours, the mixture is filtered and made up to 1,000 c.c. Ten c.c. of the sugar solution are boiled gently for 3 minutes in a 200 c.c. flask with 50 c.c. of the copper solution; the whole is rapidly cooled and titrated till colorless with a solution containing 6.55 grm. of hydroxylamine sulphate and 200 grm. of potassium thiocyanate in 2,000 c.c. Fifty c.c. of the copper solution correspond to about 60 grm. of sugar (50 mgrm. dextrose =0.1376 grm. copper). According to Jessen Hansen (*Biochem, Zeits.*, 1908, **10**, 249), the method yields good results, provided the directions are followed in every detail, particularly as regards the concentrations and temperatures at which the components of the standard solutions are mixed. The titration with hydroxylamine also necessitates very careful standardisation.

Wagner and Rinch (*Chem. Zeit.*, 1906, **30**, 38) precipitate cuprous

oxide from Fehling's solution in the usual manner, dissolve this in nitric acid and estimate the cupric nitrate with the refractometer.

Influence of Special Conditions on the Reducing Power of Sugar Solutions.—In all experiments on the reducing power of sugar on metallic solutions, it is important to operate as far as possible under constant conditions. Apparently unimportant differences, as time occupied in the experiment, amount of free alkali, presence of excess of the metallic solution, concentration of the liquid, and other conditions liable to differ with every experiment, are all factors more or less concerned in the results obtained, and rigidly accurate results thus become impossible in many cases likely to occur in the practical analysis of saccharine liquid. The irregularities due to some of the causes have been studied by Soxhlet (*Prak. Chem.*, [2] 21, 227), a very full abstract of whose original paper has been published in English by C. H. Hutchinson (*Pharm. Jour.*, [3] 1880–1, 11, 721). Soxhlet finds that the reducing power of sugar for alkaline copper solutions is only constant under exactly the same conditions, and that if the same amount of sugar act in one case on an amount of copper solution which it is just able to reduce, and in another on an excessive quantity, the reducing equivalent will in the first case be found to be considerably less than in the second. Evidently, therefore, if a solution of sugar is added by small quantities at a time to a copper solution, as in an ordinary volumetric estimation, the amount of reduction effected by the first quantities added will be greater than that produced by the last. To avoid the error due to this cause Soxhlet employs the sugar and copper solutions in the exact proportions necessary for their mutual reaction, ascertaining the volumes requisite by a series of approximating experiments.[1]

It will be seen from Soxhlet's results that dilution of the Fehling's solution very sensibly affects the reducing power exerted by the sugar. Thus, one equivalent of invert sugar in 1% solution reduces 10.1 equivalents of cupric oxide when the undiluted cupric solution is employed, but 9.7 equivalents only when Fehling's solution

---

[1]These were made by adding to a carefully measured quantity of Fehling's solution (prepared fresh daily), at the boiling point, a certain amount of a 1 or 0.5% solution of the sugar. The reaction was allowed to continue for a specified time, when the liquid was passed through a plaited filter, and a portion of the filtrate acidulated with acetic acid and tested with potassium ferrocyanide. If a reddish-brown colouration or precipitate resulted, the experiment was repeated, a somewhat larger quantity of sugar solution being employed, and so on until a measure of sugar solution was found that would exactly suffice for the decomposition of the copper solution, while if 0.1 c.c. less of sugar solution were employed a sensible quantity of copper was found in the filtrate. Hence the volume of sugar solution required was ascertained to within 0.1 c.c.

is diluted with four measures of water. It will also be observed that Soxhlet's results show a slight but very sensible difference between the reducing power of dextrose and of invert sugar.

The consideration of these differences hardly concerns the subject of commercial analysis. The analyst is recommended to standardise his own methods of manipulation very carefully by means of sugars of known purity and to operate as far as possible under absolutely constant conditions.

Reaction of Sugars with Mercury Solutions.—Several methods have been described of estimating glucoses by their reducing action on mercuric solutions, an alkaline solution of potassium mercuric cyanide being recommended by Knapp; an alkaline solution of potassium mercuric iodide by Sachsse, and a solution of mercuric acetate by Hager. The first two of these reagents have valuable qualities. They cannot advantageously replace that of Fehling for ordinary purposes, but may occasionally be applied with advantage being unequally affected by the different kinds of reducing sugars. Their use is also open to the disadvantage that mercury solutions are likewise reduced by creatin, creatinin, glycerol and, in some cases, even by alcohol (Guillaume, Gentil. *Compt. rend.*, 1881, **93b**, 338).

Knapp's mercuric solution is prepared by dissolving 10 grm. of pure dry mercuric cyanide in water, adding 100 c.c. of sodium-hydroxide solution of 1.145 sp. gr. and diluting the liquid to 100° c.c. One hundred c.c. of this solution is equivalent to 0.202 grm. dextrose in 1/2% and 0.201 grm. in 1% solution, *i. e.*, 1 grm. dextrose in 1% solution reduces 497.5 c.c. Knapp's solution.

40 c.c. of the reagent are diluted to 100 c.c., heated to boiling and the sugar solution not stronger than 0.5% is run in *as quickly as possible* until the whole of the mercury is precipitated. To determine this point a strip of filter-paper is moistened with the clear liquid and treated with hydrochloric acid and hydrogen sulphide for mercury. This method and that of Sachsse has been carefully investigated by Otto (*J. prak. Chem.*, 1881 [2], **26**, 87). ⟩

Sachsse's mercuric solution is prepared by dissolving 18 grm. of pure dry mercuric iodide in a solution of 25 grm. of potassium iodide. To this a solution of 80 grm. of potassium hydroxide is added, and the solution diluted to 1000 c.c. 40 c.c. of this solution are boiled in a basin, and a standard solution of the sugar gradually added. The end of the reaction is attained when a drop of the supernatant liquid ceases

to give a brown colour with a drop of a very alkaline solution of stannous chloride. The end of the reaction is well defined, and the results are accurate when pure dextrose or inverted sugar is worked with, though differing with each. In presence of sucrose the results are quite erroneous. By reducing the proportion of potassium hydroxide from 80 grm. to 10 grm. per 1000 c.c. Heinrich finds that glucose may be accurately determined in presence of very varying amounts of sucrose.

Soxhlet has shown that less dextrose is required the more slowly it is added and that the concentration is of considerable influence. One hundred c.c. of the solution require 0.325 grm. dextrose in 0.5 % and 0.330 grm. in 1% solution. One grm. dextrose in 1% solution reduces 302.5 c.c. Sachsse's solution.

For information respecting other modifications of these methods Lippmann's Chemie der Zuckerarten should be consulted.

Oerum (Zeit. Anal. Chem., 1904, 43, 356) recommends the following method as rapid and suitable for clinical work. The mercury reduced from Sachsse's solution is collected on a filter, washed with warm 1% hydrochloric acid and then thoroughly with water, dissolved in boiling nitric acid and titrated by decinormal ammonium thiocyanate with iron alum as indicator by Volhard's method. The solution is standardised by a known amount of dextrose.

Glassmann (Ber., 1906, 39, 503) proposes to boil the dextrose solution with a known quantity of mercuric solution previously standardised gasometrically by hydrazine sulphate. The excess of mercuric salt remaining unreduced is similarly determined.

Cane Sugar.—Sucrose, saccharose. $C_{12}H_{22}O_{11}$.

Cane sugar is found in a very large number of plants occurring both in the sap, seeds or fruits and in the milk of the coconut. Sucrose is manufactured from beet-root and the sugar cane and to a less extent from sorghum and the sugar maple.

It forms large transparent colourless crystals having the form of monoclinic prisms and familiar in commerce under the names of "sugar crystal" and "sugar candy." These crystals have a sp. gr. of 1.55 to 1.61 according to the mode of crystallisation. Cane sugar has a rotation, $[a]_D = 66.5$; $[a]^j = 73.8$.

When cautiously heated it melts at about 160° and on cooling forms

a transparent amber-coloured solid known as barley sugar. Heated at above 160° it decomposes.

Cane sugar dissolves in about half its weight of cold water, forming a very sweet viscid liquid known as syrup. (For information respecting the sp. gr. of sugar solutions see page 289).

In boiling water it is soluble in all proportions. The boiling point of an aqueous sugar solution increases with the quantity of sugar dissolved as shown in the following table. The proportion of sugar present may thus be deduced from the boiling point.

TABLE OF THE ELEVATION OF THE BOILING POINT OF SUGAR SOLUTIONS.

(Claassen-Frentzel, Deutsche Vereinzeitschrift, 1893, p. 267.)

% sugar	Elevation of the boiling point F°	% sugar	Elevation of the boiling point F°
75.	13.2	86.75	31.1
75.5	13.7	87.	31.8
76.	14.2	87.25	32.5
76.5	14.8	87.5	33.2
77.	15.3	87.75	33.9
77.5	15.8	88.	34.6
78.	16.4	88.25	35.3
78.5	16.9	88.5	36.0
79.	17.5	88.75	36.7
79.5	18.0	89.	37.5
80.	18.6	89.25	38.3
80.5	19.3	89.5	39.1
81.	19.9	89.75	39.9
81.5	20.5	90.	40.7
82.	21.2	90.25	41.5
82.5	22.0	90.5	42.4
83.	22.7	90.75	43.2
83.5	23.6	91.	44.1
84.	24.7	91.25	45.1
84.5	25.7	91.5	46.3
85.	26.8	91.75	47.7
85.5	27.9	92.	50.2
86.	29.2		
86.25	29.8		
86.5	30.4		

When subjected to prolonged boiling the sugar acquires an acid reaction and becomes in part inverted.

Cane sugar is almost insoluble in absolute alcohol; in aqueous

alcohol the solubility increases with the amount of water. The following table is due to Scheibler.

SOLUBILITY OF SUGAR IN ALCOHOL OF DIFFERENT STRENGTHS.

% alcohol	100 c.c. of the solution contain	Sp. gr. of the saturated solution
0	85.8	1.3248
5	82.4	......
10	79.4	1.2991
15	76.5	......
20	73.4	1.236
25	69.8	......
30	66.0	1.2293
35	61.6	......
40	56.7	1.1823
45	51.6	......
50	45.7	1.1294
55	39.6	......
60	32.9	1.050
65	25.6	......
70	17.8	0.9721
75	11.2	......
80	6.4	0.8931
85	2.7	......
90	0.7	0.8369
95	0.2	......
97.4	0.08	......
100	000	......

Sucrates.—Cane sugar forms definite compounds with some metallic oxides. Thus lime, magnesia, and lead monoxide dissolve with in syrup, but are completely reprecipitated by passing a current of carbon dioxide through the liquid. Lead is attacked by sugar solutions, slowly in the cold, but more quickly at a boiling heat, the lead passing into solution. Several calcium sucrates are known. The solution of calcium sucrate has an alkaline and bitter taste, and forms the *liquor calcis saccharatus* of pharmacy. On mixing syrup with a concentrated solution of barium hydroxide, a crystalline precipitate is obtained, having the composition $C_{12}H_{22}BaO_{12} = BaO$, $C_{12}H_{22}O_{11}$, or $C_{12}H_{21}(Ba.OH)O_{11}$. This compound may be recrystallised from boiling water, separating in brilliant scales resembling boric acid. Its sparing solubility in cold water has been utilised in the treatment

of saccharine juices, pure cane sugar being readily obtainable by decomposing the barium sucrate by sulphuric acid. On adding strontium hydroxide to a boiling 15% solution of sugar, the compound $C_{12}H_{20}(Sr.OH)_2O_{11}$ begins to separate, and when 2.5 molecules of strontium hydroxide have been added almost the whole of the sugar will be precipitated. The granular sucrate may be washed with hot water, and decomposed by carbonic acid. This process is now employed in recovering sugar from molasses.[1]

Crystalline compounds are also easily obtained with some sodium salts; thus there are sodium chloride compounds $C_1H_{22}O_{11}$, NaCl, $2H_2O$ and $2C_{12}H_{22}O_{11}$, $3NaCl,4H_2O$, which have a lower optical rotation than corresponds to the sugar contained in them. The sodium iodide compound $2C_{12}H_2O_{11}$, $3NaI,3H_2O$, which may be obtained in large crystals, has an optical power directly proportional to that of the contained sugar.

(For further information with regard to the sucrates, the reader is referred to Lippman's "*Chemie der Zuckerarten.*")

Detection of Cane Sugar.—Cane sugar is detected more readily by its physical properties than by its chemical reactions. The following are the leading characters of service in the recognition of cane sugar:

The sweet taste of the substance or solution.

The dextrorotatory action of the solution.

The form of the crystals.

The characteristic odour produced on heating the solid substance.

The production of saccharic and oxalic acids by the action of moderately concentrated nitric acid.

The formation of alcohol by the prolonged action of yeast on the warm solution.

The increase in the reducing power of the liquid on Fehling's test after inversion of the sugar by treatment with dilute acid, and the change in the rotatory power of the solution by inversion.

[1]For the extraction of *sucrose* from plant-products on a small scale, the fine substance should be boiled with strong alcohol, the solution filtered hot, and allowed to cool, when the cane sugar will usually crystallise out, or can be caused to do so after concentrating the solution. If *invert sugar* is also present, Peligot and Buignet recommend the following method: Add to the juice an equal measure of alcohol to prevent fermentation by keeping, filter, treat the filtrate with milk of lime in excess, and again filter. Boil the liquid when calcium sucrate separates in amount corresponding to two-thirds of the whole cane sugar present. The precipitate is filtered off, washed well, diffused in water, and decomposed by carbonic acid. The solution is filtered, evaporated at a gentle heat to a syrupy consistence, decolourised by animal charcoal, and mixed with strong alcohol till it becomes cloudy, when it is set aside to crystallise. If the solution, after treatment with carbonic acid, yields a turbid filtrate, solution of basic lead acetate is added, the liquid refiltered, and the excess of lead separated by hydrogen sulphide.

The similar change in the reducing and rotatory power of the solution by treatment with invertase. This reaction is very characteristic.

For information respecting the distinctive tests for *cane sugar*, *milk sugar*, *maltose*, and *glucoses* see page 301.

The greater number of the foregoing properties and reactions of cane sugar receive more precise recognition in the following section on the—

ESTIMATION OF CANE SUGAR.

Cane sugar may be estimated by a variety of methods, which may be conveniently classified according to the principles on which they are based.

a. **Estimation of Sugar from the Specific Gravity of the Solution.**—For the employment of this method it is, of course, essential that the solvent should be water, and that sensible quantities of foreign should be absent; if volatile, like alcohol, they may be removed by distillation. The method is constantly applied in sugar-works, not so much for ascertaining the amount of sugar in the juice as to obtain an estimate of the foreign matters associated with it; the sugar present matters being really ascertained by other methods, and a corresponding deduction made from the percentage of "apparent sugar" present. On page 289 *et seq.* full directions are given for deducing the proportions of cane sugar contained in aqueous saccharine solutions of various densities.

The percentage of sugar by weight having been ascertained, the number of pounds of sugar per imperial gallon of the syrup may be found by multiplying the sp. gr. by 1/10 of the percentage by weight, and dividing the product by 1000.

b. **Estimation of Cane Sugar by Weighing as Such.**—This method is employed in Payen's and Scheibler's methods of sugar-assaying, and in a few other cases.

c. **The estimation of cane sugar by fermentation** is fully described on page 298 *et seq.*

d. **The estimation of sucrose by its reducing action** after previous inversion is usually effected by heating it with hydrochloric acid (page 313), neutralising with sodium carbonate and estimating the resultant invert sugar by one of the processes described in the

section on the "Reducing Action of Sugars." For every 100 parts of invert sugar thus found, 95 parts of sucrose must be reckoned.

e. The estimation of cane sugar by observation of the rotatory action of its solution has already been fully described.

For the estimation of sucrose *in presence of other kinds of sugar*, methods *a, c, d,* and *e* are incapable of direct application. If employed both before and after inversion, methods *d* and *e* afford very satisfactory means, provided that no other body is present which is apt to suffer alteration in its reducing power or optical activity by heating with dilute acid. This is not always the case; but under such conditions the substitution of invertase for dilute acid, as suggested by Kjeldahl, renders it possible to effect the solution of this somewhat difficult problem (see page 315).

Estimation of Water in Commercial Sugar Products.—*Water* is estimated in granular cane sugars by exposing 5 grm. of the sample in a thin layer to a temperature of 60°, weighing every hour until there is no further loss. Twelve hours are frequently required for complete desiccation. Beet sugars and good cane sugars may be dried at 100°, two hours being sufficient. Sugars containing much glucose generally give too high a result if dried at 100°, owing to a partial conversion of the glucose into glucosan and caramel. Large-grained refined sugars absorb moisture with great facility after drying, and should be weighed between closed watch-glasses.

Some operators prefer to employ a temperature of 110° for estimating the water in sugar, by which means the time required is usually greatly shortened.

The estimation of water in treacle, beet, cane juice, and similar articles is tedious, owing to the low temperature which must be employed, and to the formation of a skin on the surface of the liquid. To avoid this 5 grm. (or other known weight) of the sample should be dissolved in water, and the solution made up to 100 c.c. 10 c.c. of this solution (=0.5 grm. of the original sample) are poured over about 12 or 15 grm. of previously ignited silver-sand, contained in a flat dish. The whole is dried at a temperature not exceeding 60° until constant, the increase in weight being due to the dry sugar in 0.5 grm. of the sample. By conducting the desiccation in a partial vacuum, from which the moisture is removed by sulphuric acid or chloride of calcium, the operation may be finished in a few hours.

The A. O. A. C. method is as follows:

1. **In Sugars.**—Dry from 2 to 5 grm. in a flat dish (nickel, platinum, or aluminum), at the temperature of boiling water, for ten hours; cool in a desiccator and weigh; return to the oven and dry for an hour. If on weighing there be only a slight change of weight, the process may be considered finished; otherwise the drying must be continued until the loss of water in one hour in not great.

2. **In Massecuites, Molasses, Honeys, and other Liquid and Semiliquid Products.**—Prepare pumice stone in two grades of fineness. One of these should pass through a 1 mm. sieve, while the other should be composed of particles too large for a millimeter sieve, but sufficiently small to pass through a sieve having meshes 6 mm. in diameter. Make the determination in flat metallic dishes or in shallow, flat-bottom weighing bottles. Place a layer of the fine pumice stone 3 mm. in thickness over the bottom of the dish, and upon this place a layer of the coarse pumice stone from 6 to 10 mm. in thickness. Dry the dish thus prepared and weigh. Dilute the sample with a weighed portion of water in such a manner that the diluted material shall contain from 20 to 30% of dry matter. Weigh into the dish, prepared as described above, such a quantity of the diluted sample as will yield, approximately, 1 grm. of dry matter. Use a weighing bottle provided with a cork through which a pipette passes if this weighing cannot be made with extreme rapidity. Place the dish in a water-oven and dry to constant weight at the temperature of boiling water, making trial weighings at intervals of 2 hours. In case of materials containing much lævulose or other readily decomposable substances, conduct the drying in vacuo at a lower temperature. In the case of very unstable material, the temperature can safely be lowered to 70°.

3. **Method for Drying Molasses with Quartz Sand.**—In a flat-bottom dish place 6 or 7 grm. of pure quartz sand and a short stirring rod. Dry thoroughly, cool in a desiccator, and weigh. Then add 3 or 4 grm. of the molasses, mix with the sand, and dry at the temperature of boiling water for from 8 to 10 hours. Stir at intervals of an hour; then cool in a desiccator and weigh. Stir, heat again in the water oven for an hour, cool and weigh. Repeat heating and weighing until the loss of water in one hour is not greater than 3 mg.

The sand used should be pure quartz. It should be digested with strong hydrochloric acid, washed, dried, and ignited, and kept in a stoppered bottle.

The amount of water present may also be obtained from the sp. gr (see page 289).

Estimation of Ash.—*The ash* of raw sugar may contain sand and other insoluble matters of mineral origin; various inorganic salts; and the non-volatile residues of the salts of various organic acids, among which may be acetic, succinic, oxalic, malic, tartaric, citric, aconitic (in cane sugar and juice only), aspartic (peculiar to beet sugar), melassic, saccharic, etc.

The most complete analysis of sugar-ash hitherto published is one by W. Wallace (*Chem. News.*, 1878, **37,** 76). The ash was derived from a Demerara cane sugar, the juice of which is supposed to have been treated with lime only. The raw sugar yielded 1.38 % of ash, an analysis of which gave the following results, K_2O, 29.10; Na_2O, 1.94; CaO, 15.10; MgO, 3.76; Fe_2O_3, 0.56; Al_2O_3, 0.65; SiO_2, 12.38; P_2O_5, 5.59; SO_3, 23.75; CO_2, 4.06; and Cl, 4.15%. Total, 101.03; less O equal to Cl, 0.93 = 100.10.

The complete incineration of raw sugar is very difficult to effect satisfactorily, the ash obtained being very fusible, or light and easily blown away; and, as it consists largely of potassium carbonate, it is very deliquescent, and hence difficult to weigh accurately. To avoid these inconveniences, it is usual to treat the sugar with sulphuric acid before igniting it, by which means the ash obtained contains the bases as the comparatively little volatile, difficultly fusible, and non-deliquescent sulphates. An allowance is made for the increased weight of the ash due to the "sulphation" by deducting 1/10 of its weight.

The method of procedure is as follows: If not already wet or viscous, moisten from 2 to 4 grm. of the sample all over with the least possible quantity of water, and then with a little pure and concentrated sulphuric acid. Heat the whole gently till the frothing ceases and the mass forms a dry cinder. Ignite the charred mass in a muffle at a very low red heat, and moisten the residue again with sulphuric acid when the ignition approaches completion. Continue the ignition at a low temperature till the carbon is wholly consumed, then heat to bright redness for 10 minutes, and weigh when cold. If *sand* or *clay* be present in sensible quantity, it must be estimated by dissolving the ash in hydrochloric acid and weighing the insoluble residue. This must be deducted from the total ash before making the correction of 1/10.

By this method, due to Scheibler, the bases being obtained as sulphates, approximate more nearly in weight to that of the organic salts

naturally present in the sugar which in the direct method are obtained as carbonates. It has also been proposed to obtain the lead salts of the organic acids by precipitation with lead acetate, decompose these and titrate the acids set free with potassium hydroxide. The potassium combination approximates closely to the actual salts of the sugar.

The A. O. A. C. give the following selection of methods:

1. Heat from 5 to 10 grm. of the material (sugar, molasses, honey) in a platinum dish of from 50 to 100 c.c. capacity at 100° until the water is expelled, and then slowly over a flame until intumescence ceases. The dish is then placed in a muffle and heated at low redness until a white ash is obtained. If the substance contains metal capable of uniting with platinum, a dish made of some other material must be used.

For soluble ash, digest the ash, obtained as above with water, filter through a gooch, wash with hot water and dry the residue at 100°.

2. Use 50 mg. of zinc oxide to 25 grm. of molasses or 50 grm. of sugar. Incorporate thoroughly by adding dilute alcohol and mixing; dry and ignite as above. Deduct the weight of zinc oxide used from the weight of ash.

3. Carbonise the mass at a low heat, dissolve the soluble salts with hot water, burn the residual mass as above, add the solution of soluble salts, and evaporate to dryness at 100°; ignite gently, cool in a desiccator and weigh.

4. Saturate the sample with sulphuric acid, dry, ignite gently, then burn in a muffle at low redness. Deduct 1/10 of the weight of the ash, then calculate the per cent.

5. Thoroughly mix 5 grm. of the material with a somewhat larger weight of pure quartz sand in a platinum dish; ignite in a muffle at a moderate red heat.

6. To avoid the correction of 1/10, as proposed by Scheibler, and 1/5, as proposed by Girard and Violette, when sugars are burned with sulphuric acid, Boyer suggests incineration with benzoic acid as giving the real quantity of mineral matter without correction.

The benzoic acid is dissolved in alcohol of 90%, 25 grm. of the acid to 100 c.c. of alcohol. 5 grm. of the sugar are weighed in a capsule and moistened with 1 c.c. of water. The capsule is heated slowly in order to caramelise the sugar without carbonizing it; 2 c.c. of the benzoic-acid solution are next added, and the capsule warmed until all the alcohol is evaporated; the temperature is then raised until the

sugar is converted into carbon. The decomposing benzoic acid produces abundant vapours, which render the mass extremely porous, especially if a circular motion be imparted to the capsule. The slow heating is continued until all the benzoic acid is volatilised. The carbon obtained is voluminous and of a brilliant black colour. The incineration is accomplished in a muffle at a low red heat. The capsule should be weighed quickly when taken from the desiccator, in order to avoid the absorption of water by the alkaline carbonates. Ammonium benzoate may be employed instead of benzoic acid, and the analyst should previously ascertain that neither the acid nor the ammonium salt leaves a residue on incineration. In addition to giving the mineral matter directly, this method permits the determination of its composition also—a matter of no small importance.

Soluble and Insoluble Ash.—Ash the material according to method 1; add water to the ash in the platinum dish, heat nearly to boiling, filter through ash-free filter-paper, and wash with hot water unti the filtrate and washings amount to about 60 c.c. Return the filter-paper and contents to the platinum dish, carefully ignite, and weigh. Compute percentages of water-insoluble ash and water-soluble ash.

Alkalinity of Ash.—The filtrate is titrated with N/10 hydrochloric acid and methyl orange. Excess of this acid is added to the insoluble ash in the platinum dish which is heated nearly to boiling, and when cool the excess is titrated with N/10 sodium hydroxide and methyl-orange. The results are usually expressed as the amount of decinormal acid required by the ash of 1 grm. of sample.

Mineral Adulterants in Ash.—Comparatively large quantities of saccharine products may be readily and quickly reduced to an ash for mineral examination without the troublesome frothing that ordinarily ensues in igniting at once with a free flame by proceeding as follows:

Mix 100 grm. of molasses, syrup, or honey, or of the confectionery solution, evaporated to a syrupy consistency, with about 35 grm. of concentrated sulphuric acid in a large porcelain evaporating dish. Then pass an electric current through it while stirring by placing 1 platinum electrode in the bottom of the dish near one side and attaching the other to the lower end of the glass rod with which the contents are stirred. Begin with a current of about 1 ampere and gradually increase to 4. In from 10 to 15 minutes the mass is reduced to a fine dry char,

which may then be readily burned to a white ash in the original dish over a free flame or in a muffle.

If an electric current is unavailable, treat in a large porcelain dish 100 grm. of the saccharine solution to be ashed, which should be evaporated to a syrupy consistency if not already in such condition, with sufficient concentrated sulphuric acid to thoroughly carbonise the mass, after which ignite in the usual manner.

Among the suspected adulterants to be looked for in the ash are salts of tin, used in molasses to bleach or lighten the colour; mineral pigments, such as lead chromate in yellow confectionery and iron oxide, the latter being sometimes used as an intensifier of or substitute for the natural colour of chocolate.

Tin in Molasses and other saccharine products.—Fuse the ash from a weighed portion of the sample with sodium hydroxide in a silver crucible, dissolve in water, and acidulate with hydrochloric acid; filter and precipitate the tin from this solution with hydrogen sulphide; wash the precipitate on a filter and dissolve it in an excess of ammonium sulphide. Filter this solution into a tared platinum dish and deposit the tin directly in the dish by electrolysis, using a current of 0.05 ampere. This current may be readily reduced from an ordinary 110-volt direct circuit by means of a series of lamps, or a rheostat may be improvised for this purpose, consisting of a long, vertical glass tube, sealed at the bottom, containing a column of dilute acid through which the current passes, the resistance being changed by varying the length of the acid column contained between two electrodes immersed therein, one of which is movable.

The following figures illustrate the average composition of the ash of raw cane and beet sugars, according to Monier:

| | Average composition of ash ||
	Cane sugar	Beet sugar
Potassium (and sodium) carbonate,	16.5	82.2
Calcium carbonate,	49.0	6.7
Potassium (and sodium) sulphate,	16.0 }	
Sodium chloride,	9.0 }	11.1
Silica and alumina,	9.5	none
	100.0	100.0

The following results by Scheibler are interesting, as showing the change produced in the weight and composition of sugar-ash by treatment with sulphuric acid:

	Beet-sugar Ash	
	Original	Sulphated
Potassium oxide,	25.65	25.65
Sodium oxide,	21.62	21.62
Calcium oxide,	6.53	6.53
Silica,	0.72	0.72
Carbon dioxide,	22.87	none
Sulphur trioxide,	17.63	58.38
Chlorine,	4.48	none
	99.50	112.90
Undetermined matters, and loss,	.50	less $\frac{1}{10}$ 11.29
	100.00	101.61

The following analyses by J. W. Macdonald (*Chem. News*, 1878, 37, 127) show the composition of the mixed sulphated ash obtained in the analysis of many samples of cane and beet sugar:

	Average sulphated ash	
	Cane sugar	Beet sugar
Potassium oxide,	28.79	34.19
Sodium oxide,	0.87	11.12
Calcium oxide,	8.83	3.60
Magnesium oxide,	2.73	0.16
Ferric oxide and alumina,	6.90	0.28
Silica,	8.29	1.78
Sulphur trioxide,	43.65	48.85
	100.06	100.06

With respect to these analyses, it may be remarked that phosphates were not sought for by Mr. Macdonald, but representative samples showed 2.90% of this in the cane sugar ash, and only 0.24% in the ash of beet sugar. In the treatment of beet-juice it is usual to employ an excess of lime, which is afterwards removed by carbon dioxide. Hence the phosphates of the juice would be precipitated almost entirely at an early stage of the manufacture. The proportion of phosphates in the ash of a sugar might perhaps furnish an indirect indication whether the article was manufactured from cane or from beet. Raw beet sugar, however, is readily distinguished from that derived from the cane by the appearance, flavor, and the small proportion of dextrose, owing to the destruction of the greater part by the employment of a large excess of lime.

According to Landolt, in the case of beet sugars, the ratio between the potassium carbonate and the amount of organic salt is approximately as 1:2 which becomes 1:1.54 if the potassium is estimated as sulphate.

Laugier (*Compt. rend.*, 1878, **87**, 1088) claims to reconstruct the original salts in the following manner. To a sample, dilute sulphuric acid is cautiously added to set free the organic acids which are then extracted with ether. Half this ethereal solution is added to the ash derived from another sample of the sugar of half the weight, evaporated down upon it and weighed.

As any clay or sand contained in a sample of sugar has no prejudicial effect on the refining process, it is sometimes desirable to eliminate such extraneous matters before determining the ash proper. This is done by dissolving a known weight of the sample in water, making the solution up to a known volume, filtering through a dry filter, evaporating one-half of the filtrate to dryness, moistening the residue with sulphuric acid, and igniting in the usual way.

Extractive Matters. Organic Matters not Sugar.—In ordinary commercial analyses of sugars, the sum of the sucrose, dextrose, ash, and water is subtracted from 100.00, and the difference called "organic or undetermined matters." Under the last denomination are included many substances, of which the chief are: organic salts of the bases found in the ash; organic bases, such as asparagine and betaine; gummy and pectous bodies; proteins and enzymes; and insoluble organic matters, such as particles of cane. Some of these impurities have no interest for the sugar refiner, but others are very injurious. Thus the gummy matters interfere with the process of crystallisation, and the proteins tend to induce fermentation.

Although for most commercial purposes the estimation of these substances by difference is sufficient, the method is open to the objection that all the errors of the analysis are thrown on the organic matters, and that such a method makes no distinction between the harmless and injurious bodies comprised among the "organic matters not sugar." Hence even rough methods of obtaining a further knowledge of the nature and amount of these substances have an occasional value.

Walkoff obtains a comparative estimate of the organic matters in beet products by precipitating the solution of 5 grm. of the sugar in 200 c.c. of warm water by a solution of 2 grm. of pure tannin in 1000 c.c. The tannin solution is added from a burette, and samples of the liquid

filtered from time to time, and the filtrate tested with ferrous sulphate, which gives a dark colour as soon as the tannin has been added in excess. The tannin is said to precipitate 1/6 of its weight of organic matters, but the process is chiefly valuable as a test for the comparative purity of different specimens. Asparagine is not estimated in this process. The sugar solution should be perfectly neutral.

Another comparative method consists in precipitating the solution of sugar with a slight excess of basic lead acetate, and weighing the precipitate produced, or the organic matters recoverable from it by decomposing it with sulphuretted hydrogen.

Invert sugar in raw sugar may be estimated by Fehling's, Knapp's, or Sachsse's method. Fehling's solution employed gravimetrically requires a somewhat longer time than some of the volumetric methods. On the other hand, the latter require that the solution shall be tolerably free from colour.

Dextrose, in a proportion greater than one-half of the total invert sugar, is not a normal constituent of commercial cane sugar, but is sometimes added as an adulterant.

Sucrose may be determined in raw sugar by the polarimetric method. In samples containing but little invert sugar the original reading will be sufficiently accurate for commercial purposes, but in other cases it should be supplemented by Clerget's inversion-process (page 312).

Assay and Valuation of Raw Sugar Products.—Sugars, whether raw or refined, which are fit for direct consumption are generally valued for their appearance, colour, etc., rather than according to the percentage of sugar present. But when bought for the purpose of refining it is important to know not only how much sucrose the sample actually contains, but also the available amount of crystallisable sugar.

Two samples of sugar containing the same percentage of sucrose often differ considerably in their yield of crystallisable sugar when refined. This is attributable to differences in the nature and quantity of the impurities, which either tend to destroy the sucrose by inversion or prevent its crystallisation. These considerations resulted in the adoption of the assumption that each unit of ash prevents five units of cane sugar from crystallising, and that each unit of invert sugar prevents the crystallisation of an equal weight (or according to some practices twice its weight) of cane sugar. Hence a deduction equal to (twice) the percentage of invert sugar found *plus* 5 times the weight of the ash, must be made from the content of cane sugar found

by analysis, in order to ascertain the percentage of net obtainable or crystallisable sugar in the sample.[1] This percentage of crystallisable sugar is called the "refining value" of the sample. The results of the above calculation are not always in strict accordance with the truth, though for beet sugar the variations are not great of late years the proportion of organic non-sugar to ash is said to have increased. When the ratio of ash to organic non-sugar is about 2:1 the yield actually obtained is less than that calculated by deducting five times the weight of the ash. Schultz considers that the out-turn of refined beet sugar is equal to the total sugar *minus* twice the amount of total soluble impurities.

A very convenient and instructive method of assaying a *juice*, *syrup*, or *molasses* is to ascertain the ratio which exists between the percentage of sugar as determined by the polarimeter and as deduced from the sp. gr. of the liquid. The difference between the two results is the percentage of "solids not sugar," and though the non-identity of the solution sp. gr. of these matters with that of sugar prevents the method from giving really accurate results, it affords a simple and practical means of judging of the relative purity of saccharine liquids, and calculating the amount of crystallisable sugar obtainable therefrom. The percentage of "apparent sugar," or total solids in the liquid, can be deduced from the table of sp. gr. on page 289, and this figure multiplied by the sp. gr. of the solution gives the number of grm. of total solids per 100 c.c. This result may also be obtained from the formulæ on page 290, but for very strong saccharine liquids, such as molasses, the use of the table is preferable. From the contents of the liquid in total solids thus found there is subtracted the weight (grm.) of sugar per 100 c.c. found by the polarimeter, when the difference is the "solids not sugar" per 100 c.c. The percentage of real sugar contained in 100 parts of total solids, or "apparent sugar," is called the "apparent-purity-coefficient" of the juice.

A rapid approximate valuation may be obtained by making a perfectly saturated solution of the sample in water at 17.5°, and ascertaining the sp. gr. of the liquid. In the case of pure sucrose this will

[1] A commission appointed by the French Government recommended the following plan of valuing raw sugars, which was the officially recognised method in France, though it has not met with general acceptance in other countries, as its indications are liable to be erroneous in the case of cane sugar. From the percentage of sucrose shown by the polarimeter is substracted the sum of:
a. *Four* times the weight of the ash. (By "ash" is meant sulphated ash multiplied by 0.8.) b. *Twice* the invert sugar when the latter reaches 1 per cent.; or a weight *equal* to the invert sugar when the latter is between 0.5 and 1 %. When the invert sugar is below 0.5 % the correction b is neglected. 1.5 % for waste in refining.

not exceed 1330.0; but the sp. gr. increases with the proportion of foreign substances. The following table is given by E. Anthon (*Jahresb.*, 1868, 957):

Specific gravity of saturated solution	Percentage composition of solution saturated at 17.5°		
	Sugar	Other substances	Water
1330.0	66.66	0.00	33.34
1332.2	64.85	2.66	32.49
1338.4	63.70	5.29	31.01
1344.6	62.65	7.76	29.68
1350.9	61.42	10.13	28.45
1357.2	60.28	12.48	27.24
1363.6	59.14	14.67	26.19
1370.0	58.00	16.82	25.18
1376.4	56.85	18.87	24.28
1382.9	55.70	20.77	23.53
1389.4	54.56	22.59	22.85
1395.9	53.42	24.36	22.22
1402.5	52.28	25.98	21.74
1409.2	51.14	27.56	21.30
1415.9	50.00	29.00	21.00

In the case of cane-juice products, the "solids not sugar" are found in practice to prevent the crystallisation of an equal weight of sugar, but 1 % of the "solids not sugar" from beet-root will prevent the crystallisation of 1.2% of sugar. Hence a sugar-cane product having an apparent-purity-coefficient of less than 50 cannot be made to yield any crystallisable sugar, and the same is true of a beet-root product having a coefficient somewhat greater than this.[1] By removing the salts even molasses can be made to yield considerable crystallised sugar.

Adulterations of Commercial Sucrose.—Sugar may contain woody fibre from the crushed cane, much gritty sand, fungus spores and when in bulk all kinds of make-weights. The presence of sand and earthy matter is, of course, indicated by an excessive proportion of ash and the incomplete solubility in water.

Ultramarine is now frequently added to refined sugars to correct

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[1] Thus, if a beet-juice has a coefficient of 79, the residue on evaporation will contain 79% of sugar and 21 of impurities. 21×1.2 = 25.2, which deducted from 79 leaves 53.8 as the percentage of the total solids obtainable in the form of crystallisable sugar.

any yellowish tint.   It may be easily detected by dissolving the sugar in cold water and allowing the suspended matter to settle.

Fungus spores are objectionable from the extreme rapidity with which, under suitable conditions, they develop into a spreading vegetable growth, especially in presence of nitrogenous matter.   Such sugar is apt to undergo fermentation and turn sour, and preserves made with it soon spoil.

The **Acarus sacchari**, or sugar-mite, is a small animal closely resembling the itch-insect, and, like it, capable of burrowing under the skin and producing an irritating pustular disease called the "grocer's itch," which attacks those employed in handling raw sugars.

Starch-sugar ("Glucose") is employed as an adulterant of the lower grades of refined cane sugar.   The starch-sugar used is commonly a highly-converted kind, as the other varieties are too deliquescent to be suitable for the purpose.   Anhydrous dextrose is sometimes employed, and the adulterated sugars generally contain less moisture than the genuine sugars of the same grades, which are known as "coffee sugars," and are always sold moist.   The proportion of starch-sugar employed as an adulterant is usually about 20%.

If the sense of taste be first deadened by placing a pinch of pure powdered cane sugar on the tongue, and then, while the taste remains, a portion of the suspected sample tested in the same way, the bitterness of starch-sugar will be distinctly perceived if the specimen under examination be adulterated.

If the sample suspected to contain starch-sugar is placed in a beaker and stirred for a few seconds with rather less than its own weight of cold water, any hydrated dextrose will be seen floating in the liquid as white specks resembling crushed wheat.   Anhydrous dextrose does not behave similarly, the crystals appearing as translucent as cane sugar.

When examined by Fehling's solution, genuine coffee sugar will rarely cause a reduction greater than corresponds to 5% of dextrose, while a sugar adulterated with the usual propo.tion of starch-sugar will show a reduction corresponding to about 20% of dextrose.   Owing to the irregular composition of commercial starch-sugar, the proportion of it present in coffee sugar cannot be deduced with accuracy from the reducing power of the sample.

The same difficulty arises when an attempt is made to deduce the extent of adulteration from the optical activity of the sample; and, as

commercial starch-sugar undergoes more or less change in its rotatory power by inversion with dilute acid, Clerget's method cannot be employed for the estimation of the sucrose present. Nevertheless, the polarimeter affords qualitative results of great value, and allows the fact of adulteration to be established beyond the possibility of doubt.

Some samples of coffee sugar adulterated with starch-sugar exert a rotation corresponding with upwards of 100% of cane sugar, owing to the high rotatory power of maltose and dextrin. Such a result is sufficient to establish the presence of starch-sugar. In cases of adulteration by more highly-converted starch-sugar, the direct polarimetric test will fail to indicate the existence of adulteration, but the fact will become manifest on inversion, which process will fail to produce the same change in the polarimetric reading that would ensue if only cane sugar and a small proportion of invert sugar are present.

Casamajor has proposed to utilise the fact that dextrose has a higher optical activity where freshly dissolved than after some time. The standard weight of sugar is dissolved in cold water, made up to 100 c.c. and the solution examined in the polarimeter with as little delay as possible. If the sugar is genuine, the rotation first observed will remain unchanged for any length of time, but if starch-sugar be present the rotatory power will gradually diminish. A sample examined by Casamajor showed 100.4 when first observed. In 15 minutes, the sugar-indication had fallen to 94.3; to 91.6 in 30 minutes; to 90.02 in 1 hour; to 89.7 in 3 hours; and to 89.3 in 5 hours, when it became stationary. After inversion, the sugar-indication was 72.7 (*Chem. News*, 1883, **48**, 252).

Molasses, Treacle and Golden Syrup.—These by-products of the sugar industry should consist essentially of sucrose and invert sugar. They are often adulterated with glucose syrup. The production tion of molasses is due to the long-continued heating of the saccharine juice, but the quality varies with the nature and culture of the sugar-yielding plant, and with many other circumstances. "Refiners' molasses," the syrup obtained in the refining of sugar, retains a considerable amount of sucrose, the proportion being about 35% in cane-sugar molasses, and as much as 50% in that from beet-root. This is prevented from crystallising by the impurities present in the raw sugar. The molasses from raw cane sugar contains a considerable percentage of invert sugar, from which beet-root molasses is comparatively free, but the latter contains raffinose, aspartic acid,

and some other substances. The proportion of salts contained in beet-root molasses is usually 10 to 14%, whereas refiners' treacle from raw cane sugar rarely contains half that proportion.[1]

The following analyses show the general composition of molasses:

	Su-crose	Invert Sugar	Ash	Wa-ter	Organic matters other than sugar	Authority
Sugar-cane Products:						
Green syrup	62.7	8.0	1.0	27.7	0.6	W. Wallace.
Golden syrup	39.6	33.0	2.5	22.7	2.8	W. Wallace.
Treacle	32.5	37.2	3.5	23.4	3.5	W. Wallace.
Molasses	48.0	18.0	1.4	31.1	18.0	W. Wallace.
Molasses, average	35	10	5	20	10	J. H. Tucker.
Molasses, refiners'	37.5	..	..	25	..	Casamajor.
Beet-root Products:						
Molasses	50.9	1.1	12.9	19.0	16.1	Houghton Gill.
Molasses, average	50	..	10	20	20	Wigner and Harland.
Molasses, average	55	trace	12	20	13	J. H. Tucker.
Molasses, average	49.4	..	..	17.1	..	Payen.

Bodenbender found an average of 1.5% of nitrogen in beet-root molasses, of which nearly 1% existed as betaine and proteins, and nearly the whole of the remainder as aspartic and glutamic acids and asparagine.

Vanillin has been recently recognised in beet-sugar molasses and may even be extracted from many samples of raw sugar by simple agitation with ether.

The analysis of molasses and syrups may be effected by the methods employed for raw sugar, but certain modifications are rendered necessary by the character of the substance.

Water may be estimated as described on page 343. When no great accuracy is required, an approximation can be obtained by taking the sp. gr. of the syrup, but, owing to the salts and extractive matters of molasses having different solution-densities from that of sugar, the results are seriously vitiated in many cases. The water may also be estimated by Wiley's method.

The ash and organic matter not sugar may be ascertained as in raw sugars (pages 345 to 348).

Dextrose may be estimated in the usual way by Fehling's solution. This is not seriously affected by the presence of the other organic matters, unless a very accurate result is required, in which case the

[1] The United States standards of purity require that molasses contains not more than 25% of water and 5% of ash; treacle contains not more than 25% of water and 8% of ash.

solution must be clarified by means of lead, and the excess of lead re-
moved as described on page 311. The solution of dextrose should be
dilute.

The estimation of the sugars and more especially of the sucrose
in cane molasses has been the subject of much discussion. Esti-
mation of the sucrose by direct polarisation is impossible on account of
the large amount of reducing sugars present and resource is had to the
Clerget method, the inversion being best performed by invertase.
When acid is used the polarisation of the reducing sugars is said not to
be affected, but some observers claim that the polarisation of the
lævulose is not the same in neutral as in acid solutions. In any case,
the error is only very slight and can be obviated by using invertase to
effect inversion.

When cane-sugar molasses is fermented, the quantity of alcohol pro-
duced is much less than that calculated from the amount of sucrose
and reducing sugars present. Harker (J. Soc. Chem. Ind., 1906, 25,
831) has shown that this is due to the formation of non-fermentable
sugars by the action of acids on the molasses and that the quantity
produced by the action of acids is very much greater than by that of
invertase. The excess of reducing bodies produced by acid is, there-
fore, not derived from sucrose. If this result be accepted, an additional
justification of the use of invertase for inversion is obtained.

Alternative to the use of the polarimeter is the determination of the
reducing sugars with Fehling solution before and after inversion with
invertase.

In the molasses from beet sugar (more especially) certain optically
active substances other than sugar are present. Of these, malic, meta-
pectic, and alkaline solutions of aspartic acid are lævorotatory, besides
invert sugar and beet gum. Dextran, asparagine, glutamic acid, and
acid solutions of aspartic acid exercise a right-handed rotation. These
interfering substances, of which the dextran and beet gum are the most
optically active, tend in great measure to neutralise the effect of each
other. The optical effect of asparagine is said to be completely neu-
tralised by adding 10% of acetic acid to the solution filtered from
the lead precipitate.

If one-half the standard weight of syrup be weighed out, treated with
1 c.c. of lead solution, and the mixture made up to 50 c.c. by absolute
alcohol and filtered, all the asparagine, aspartic acid, malic acid,
beet gum, and dextran remain in the precipitate, while the presence of

the alcohol in the filtrate is said to neutralise the rotation due to the invert sugar.

Sugar Confectionery.—Analysis of sweets is generally a question of the detection of poisonous colouring materials. The percentage of sugar present may be estimated in the usual way and the presence of starch-sugar ascertained as described (see page 354). Starch-sugar is very extensively employed in the manufacture of confectionery.

Essences may be dissolved out by petroleum spirit and identified by their odour; those now used are often artificial.

Treatment of the colouring matter with alcohol, with water and with bleaching powder quickly characterises it as organic or inorganic in nature.

Among the red colouring matters of sugar confectionery, red lead and vermilion have been observed, but in most cases harmless organic pigments are employed.

Lead chromate has been employed as a yellow colouring agent. Greens have been found to be produced by a mixture of lead chromate and prussian blue, and copper arsenite, and other cuprous pigments have also been met with. The blue mineral colouring matters may be of prussian blue or ultramarine. The detection of the injurious colouring matters in confectionery belongs to mineral analysis, and requires no detailed description here.

Candies and confections are now almost invariably coloured with coal-tar products, prepared especially for the purpose and free from metallic impurities. In most cases very small amounts of colour are used.

Under a regulation issued in accordance with the provisions of the U. S. (Federal) food law the following eight colours are permitted in candies and confections, the manufacture and sale of which are within the jurisdiction of the law. The numbers refer to Schultz & Julius' Systematic Survey of Organic Coloring Matters (translated by Green):

107 Carmosine B.
56 Scarlet 40.
517 Eosin B. C.
85 Orange G.
4 Yellow F. Y.
435 Acid green GG.
692 Indigotin.

The manufacturer of the said dyes is required to guarantee that they really are what they are represented to be, that they are not mixtures and that they do not contain harmful impurities.

Sugar-cane and Beet Juices.

The juice obtained by crushing and pressing the sugar-cane[1] has usually a sp. gr. of 1.070 to 1.090, but has been met with as low as 1.046 and as high as 1.110. It is an opaque, frothy, yellowish-green liquid. On filtration it yields a pale yellow fluid, which is nearly pure syrup, the greenish scum containing chlorophyll, a peculiar wax called cerosin,

[1] The following analyses show the general composition of the sugar-cane:

Locality and kind of cane	Water	Sugar	Woody fibre	Salts	Authority
Martinique	72.1	18.0	9.9		Peligot.
Guadaloupe	72.0	17.8	9.8	0.4	Dupuy.
Havana	77.0	12.0	11.0	..	Casaseca.
Cuba	65.9	17.7	16.4	..	Casaseca.
Mauritius	69.0	20.0	10.0	1.0	Icery.
Ribbon cane	76.73	13.39	9.07	.39	Avequin.
Tahiti	76.08	14.28	8.87	.35	Avequin.

The following is a more detailed analysis, by Payen, of Otaheite cane at maturity:

Water	71.04
Sugar	18.00
Cellulose, ligneous matter, pectin, and pectic acid	9.56
Proteins	0.55
Cerosin; red, green, and yellow colouring matters; fatty matter; resins; essential oil; aromatic matter; and a deliquescent substance	0.37
Insoluble salts, 0.12; soluble, 0.16, consisting of phosphates, sulphates, chlorides, oxalates, acetates, malates,	0.28
	99.80

According to Casaseca, the lower portions of the sugar-cane are the richest in sugar, the centre being of about the average composition. This is shown by the following analysis by Gill of carefully sampled good average cane from the Aska district, Madras:

	A	B	C
	Two feet top	Two feet middle	Two feet root
Megass proper	7.63%	8.47%	8.30%
Juice	92.37%	91.58%	91.70%
Containing, cane sugar	10.63%	13.31%	13.37%
Containing, invert sugar	2.64%	1.51%	1.54%

protein matters, fibre, and a considerable proportion of mineral matter. The pure or nearly colourless juice from which the green matter has been separated contains in 100 parts: water, 81.00; sugar, 18.20; organic matters precipitated by lead salts, 0.45; and mineral matters, 0.35.

The sp. gr. of the juice from the white beet[1] is usually between 1060 and 1070, occasionally reaching 1078. Beet juice contains a large amount of foreign matters in proportion to the sugar, a fact that renders the manufacture of sugar from beet-root much more troublesome than from cane. The average percentage composition of expressed beet juice is approximately:—water, 82.68; sugar, 11.25; other organic matters, 1.47; and mineral matters, 0.67.

The analysis of cane and beet juices may be effected by the method described under "Molasses" and "Raw Sugar."

The methods in vogue for the estimation of sugar in the beet, which is first reduced to a pulp by a suitable press, are as follows: (a) Warm alcohol extraction (Sickel-Soxhlet); (b) and (c) hot or cold alcohol digestion; (d) hot aqueous digestion; (e) cold water digestion (Pellet).

The expressed juice had the following composition:

	A	B	C
Cane sugar	11.51	14.55	14.58
Invert sugar	2.86	1.65	1.68
Ash	.33	.28	.25
Unknown	.50	.92	.49
Apparent solids	15.20	17.40	17.00
Water	84.80	82.60	83.00
	100.00	100.00	100.00

The megass referred to above contains little but woody fibre, as the sugar is extracted in the Aska district by the diffusion process. Ordinary megass or mill-trash after passing the rollers retains 8 or 10 % of sugar and 50 % of water.

The ash of the sugar-cane contains about 50 of silica, 5 to 8 of phosphoric acid, and different proportions of potassium. Sodium appears to be a constant constituent.

[1] The following is an analysis by Payen of the white or sugar beet:

Water,	82.7
Sugar,	11.3
Cellulose,	0.8
Proteins.	1.5
Fatty matter,	0.1
Pectin matters, asparagine, aspartic acid, betain; oxalates, nitrates, phosphates,	3.7
	100.1

For the cold solution processes a more finely divided pulp is required. The alcohol extraction is perhaps the most accurate, Pellet's process the most widely used in factories.

Alcohol Extraction.—The normal weight of beet pulp with the addition of 3 cm. of lead acetate is extracted with absolute alcohol in a Soxhlet until the sugar has all gone into solution. The alcoholic solution is made up to 100 c.c. and polarised in a 200 mm. tube when the percentages of sugar is read off directly.

Alcohol Digestion.—Twice the normal weight of pulp with 3 or 4 c.c. of lead acetate is heated 15 to 20 minutes with 90% alcohol; the flask is cooled to 20° and filled up to the mark 201.2 c.c., the additional volume of 1.2 c.c. being to correct for the volume of the marc and the lead precipitate. The solution is filtered and polarised as usual. The cold extraction with alcohol is performed in a similar flask, likewise the hot aqueous digestion.

Pellet's cold aqueous digestion method consists in taking 26.0 grm. of very finely pulped beet, 5 to 6 c.c. lead acetate, adding cold water nearly to the mark, shaking vigorously, making up to 200.6 c.c. and filtering. The liquid is polarised and the sugar value obtained doubled to give the percentage in the beet pulp.

Davoll (*J. Amer. Chem. Soc.*, 1906, **28**, 1606–1611) proposes as a rapid modification of the above to take 52 grm. of pulp, make up with lead acetate and water to 209.2 grm. in a beaker instead of in a flask.

MALTOSE

is the chief product of degradation of starch and also occurs in the leaves of some plants. It usually occurs in fine crystalline needles of the hydrate $C_{12}H_{22}O_{11},H_2O$. The amorphous anhydride is very hygroscopic. A convenient method for the preparation of pure maltose is given by Baker and Day. (*Analyst*, 1908, **33**, 393). Maltose is hydrolysed to two molecules of dextrose when heated with dilute acids, but is far more stable than is sucrose (see page 296). Hydrolysis takes place more rapidly under the influence of a specific enzyme, *maltase*. This enzyme affords an absolute means of identifying maltose; it is contained in dried yeast (see Invertase, page 314) and may be prepared by extracting this for an hour with 20 times its weight of water at about 20° and filtering. A few c.c. of this extract are added to 50 c.c. of a 5% solution of the carbohydrate under examination, a little toluene is added and the whole is incubated in a

closed flask at 37° for 24 hours. The optical activity or cupric reducing power may be taken before and after the action, change denoting the presence of maltose. (See also Barfoed's reagent, page 333.)

Maltose is not fermented directly by yeast, but is first hydrolysed to dextrose by the maltase present in most yeasts, and this dextrose is converted into alcohol and carbon dioxide by the yeast. Some species of yeast—*S. marxianus, S. exiguus, S. Ludwigii, W. anomala, W. Saturnus* (see E. F. Armstrong, *Proc. Roy. Soc.*, 1905, 76 B, 600)— do not contain maltase and are therefore incapable of fermenting maltose. Use may be made of these yeasts to detect traces of dextrose or lævulose present in maltose, as these sugars will form carbon dioxide when fermented, whilst the maltose remains unattacked.

Maltose has a value for $[a]_D = 138°$ (see page 305) and shows birotation (see page 315); the rotation of a freshly dissolved specimen increases on keeping or on the addition of a trace of ammonium hydroxide. Maltose resembles the glucoses in its power of reducing hot Fehling's solution without previous inversion, but the amount of cuprous oxide precipitated is only 62% of that reduced by an equal weight of dextrose.

The reducing power of maltose is 60.8 according to Brown and Heron, assuming 1038.6 to be the sp. gr. of a solution of maltose containing 10 grms. per 100 c.c. Correcting this for the true sp. gr. found by them (1039.3) the value of K becomes 61.9 (*Journ. Chem. Soc.*, 1879, 35, 618).

Soxhlet states that the cupric reducing power (K) is 61 when the maltose is contained in a 1% solution, and the Fehling reagent is undiluted and employed in the exact proportion necessary; 64.1 when the copper solution is previously diluted with 4 volumes of water; and 65.3 when twice as much of this diluted Fehling's solution is used as is required for the reaction (*J. pr. Chem.*, 1880, [2], 21, 227).

When a solution of maltose is treated with a volume of Fehling's solution sufficient for its oxidation, the mixture heated, and the cuprous oxide filtered off in the usual way, a solution is obtained which, if acidulated with hydrochloric acid and heated, acquires the property of reducing an additional quantity of Fehling's solution. This second reduction is somewhat more than half the first, so that the two together approach to the reducing power of dextrose. A similar behaviour is exercised by milk sugar (Herzfeld, *Annalen*, 1883, 220, 206).

According to I. Steiner, the reducing action of maltose on Pavy's

ammoniacal cupric solution is the same as upon the ordinary Fehling's reaction. Thus, 20 c.c. of Fehling's solution will require the same volume of maltose solution for its reduction, whether used direct or previously mixed with 40 c.c. of strong ammonia, and titrated as described on page 331 (Yoshida, *Chem. News*, 1881, **43**, 29). On the other hand, the addition of more sodium hydroxide in presence of ammonia increases the oxidising power of the copper solution to a notable extent (*Chem. News*, 1880, **42**, 45).

The estimation of maltose in cases of practical interest, viz.: in starch-sugar and brewing materials, is described elsewhere. The general methods already described are all applicable to maltose. 1 grm. of maltose in 1% solution corresponds to 317.5 c.c. of Knapp's and 197.6 c.c. of Sachsse's solution.

The A. O. A. C. method of estimating maltose is as follows:

Place 50 c.c. of the mixed copper reagent in a beaker and heat to the boiling point. While boiling briskly, add 25 c.c. of the maltose solution containing not more than 0.250 grm. of maltose and boil for 4 minutes. Filter immediately through asbestos and ascertain the amount of copper reduced by one of the methods given, page 323. Obtain the weight of maltose equivalent to the weight of copper found from the following table:

TABLE FOR THE ESTIMATION OF MALTOSE.
(According to Wein.)

Milli-grams of copper	Milli-grams of cuprous oxide	Milli-grams of maltose	Milli-grams of copper	Milli-grams of cuprous oxide	Milli-grams of maltose	Milli-grams of copper	Milli-grams of cuprous oxide	Milli-grams of maltose
31	34.9	26.1	51	57.4	43.5	71	79.9	61.0
32	36.0	27.0	52	58.5	44.4	72	81.1	61.8
33	37.2	27.9	53	59.7	45.2	73	82.2	62.7
34	38.3	28.7	54	60.8	46.1	74	83.3	63.6
35	39.4	29.6	55	61.9	47.0	75	84.4	64.5
36	40.5	30.5	56	63.0	47.8	76	85.6	65.4
37	41.7	31.3	57	64.2	48.7	77	86.7	66.2
38	42.8	32.2	58	65.3	49.6	78	87.8	67.1
39	43.9	33.1	59	66.4	50.4	79	88.9	68.0
40	45.0	33.9	60	67.6	51.3	80	90.1	68.9
41	46.2	34.8	61	68.7	52.2	81	91.2	69.7
42	47.3	35.7	62	69.8	53.1	82	92.3	70.6
43	48.4	36.5	63	70.9	53.9	83	93.4	71.5
44	49.5	37.4	64	72.1	54.8	84	94.6	72.4
45	50.7	38.3	65	73.2	55.7	85	95.7	73.2
46	51.8	39.1	66	74.3	56.6	86	96.8	74.1
47	52.9	40.0	67	75.4	57.4	87	97.9	75.0
48	54.0	40.9	68	76.6	58.3	88	99.1	75.9
49	55.2	41.8	69	77.7	59.2	89	100.2	76.8
50	56.3	42.6	70	78.8	60.1	90	101.3	77.7

TABLE FOR THE ESTIMATION OF MALTOSE.—Continued.

Milligrams of copper	Milligrams of cuprous oxide	Milligrams of maltose	Milligrams of copper	Milligrams of cuprous oxide	Milligrams of maltose	Milligrams of copper	Milligrams of cuprous oxide	Milligrams of maltose
91	102.4	78.6	146	164.4	127.8	201	226.3	177.0
92	103.6	79.5	147	165.5	128.7	202	227.4	177.9
93	104.7	80.3	148	166.6	129.6	203	228.5	178.7
94	105.8	81.2	149	167.7	130.5	204	229.7	179.6
95	107.0	82.1	150	168.9	131.4	205	230.8	180.5
96	108.1	83.0	151	170.0	132.3	206	231.9	181.4
97	109.2	83.9	152	171.1	133.2	207	233.0	182.3
98	110.3	84.8	153	172.3	134.1	208	234.2	183.2
99	111.5	85.7	154	173.4	135.0	209	235.3	184.1
100	112.6	86.6	155	174.5	135.9	210	236.4	185.0
101	113.7	87.5	156	175.6	136.8	211	237.6	185.9
102	114.8	88.4	157	176.8	137.7	212	238.7	186.8
103	116.0	89.2	158	177.9	138.6	213	239.8	187.7
104	117.1	90.1	159	179.0	139.5	214	240.9	188.6
105	118.2	91.0	160	180.1	140.4	215	242.1	189.5
106	119.3	91.9	161	181.3	141.3	216	243.2	190.4
107	120.5	92.8	162	182.4	142.2	217	244.3	191.2
108	121.6	93.7	163	183.5	143.1	218	245.4	192.1
109	122.7	94.6	164	184.6	144.0	219	246.6	193.0
110	123.8	95.5	165	185.8	144.9	220	247.7	193.9
111	125.0	96.4	166	186.9	145.8	221	248.7	194.8
112	126.1	97.3	167	188.0	146.7	222	249.9	195.7
113	127.2	98.1	168	189.1	147.6	223	251.0	196.6
114	128.3	99.0	169	190.3	148.5	224	252.4	197.5
115	129.6	99.9	170	191.4	149.4	225	253.3	198.4
116	130.6	100.8	171	192.5	150.3	226	254.4	199.3
117	131.7	101.7	172	193.6	151.2	227	255.6	200.2
118	132.8	102.6	173	194.8	152.0	228	256.7	201.1
119	134.0	103.5	174	195.9	152.9	229	257.8	202.0
120	135.1	104.4	175	197.0	153.8	230	258.9	202.9
121	136.2	105.3	176	198.1	154.7	231	260.1	203.8
122	137.4	106.2	177	199.3	155.6	232	261.2	204.7
123	138.5	107.1	178	200.4	156.5	233	262.3	205.6
124	139.6	108.0	179	201.5	157.4	234	263.4	206.5
125	140.7	108.9	180	202.6	158.3	235	264.6	207.4
126	141.9	109.8	181	203.8	159.2	236	265.7	208.3
127	143.0	110.7	182	204.9	160.1	237	266.8	209.1
128	144.1	111.6	183	206.0	160.9	238	268.0	210.0
129	145.2	112.5	184	207.1	161.8	239	269.1	210.9
130	146.4	113.4	185	208.3	162.7	240	270.2	211.8
131	147.5	114.3	186	209.4	163.6	241	271.3	212.7
132	148.6	115.2	187	210.5	164.5	242	272.5	213.6
133	149.7	116.1	188	211.7	165.4	243	273.6	214.5
134	150.9	117.0	189	212.8	166.3	244	274.7	215.4
135	152.0	117.9	190	213.9	167.2	245	275.8	216.3
136	153.1	118.8	191	215.0	168.1	246	277.0	217.2
137	154.2	119.7	192	216.2	169.0	247	278.1	218.1
138	155.4	120.6	193	217.3	169.8	248	279.2	219.0
139	156.5	121.5	194	218.4	170.7	249	280.3	219.9
140	157.6	122.4	195	219.5	171.6	250	281.5	220.8
141	158.7	123.3	196	220.7	172.5			
142	159.9	124.2	197	221.8	173.4			
143	161.0	125.1	198	222.9	174.3			
144	162.1	126.0	199	224.0	175.2			
145	163.2	126.9	200	225.2	176.1			

Maltose may be distinguished from dextrose by its neutral behaviour towards copper-acetate solution (Barfoed's reagent) and by the solubility of its osazone in hot water.

On heating with phenylhydrazine at the temperature of the water-bath, dextrose or lævulose gives a precipitate of the phenylosazone after 10 minutes, but maltose forms a precipitate only on cooling the solution after an hour's heating. The osazones of the two sugars can thus be separated easily from a solution containing both sugars. Maltosazone is soluble in about 75 parts of hot water, whereas glucosazone is almost insoluble. When testing a freshly prepared mixture of osazones in this manner, it is essential to first wash them thoroughly with water and benzene so as to remove products which tend to make glucosazone appear soluble. Maltosazone is soluble in a cold mixture of equal parts of water and acetone.

Baker and Dick (*Analyst*, 1905, **30**, 79) state that small quantities of maltose may be estimated with a fair degree of accuracy by taking the reducing power before and after inversion. They consider that, depending on the osazone reaction alone, it is impossible to detect less than 15% of maltose in admixture with dextrose. Small quantities of maltose may be identified after first removing the dextrose by fermentation with *S. marxianus*.

LACTOSE.

Milk sugar reduces Fehling's copper solution, the reducing power being roughly half that of dextrose. It rapidly reduces ammoniacal silver nitrate. The osazone is soluble in boiling water and it may thus be detected in presence of the glucoses or galactose. Characteristic of lactose and galactose is the formation of mucic acid when oxidised by nitric acid. Use is often made of the low solubility in water and facility of crystallising to identify lactose.

Milk sugar has a value for $[a]_D = 52.7°$ and exhibits mutarotation (see page 315). Wiley uses $[a]_D = 52.53°$.

In practice the estimation of milk sugar is required simply in milk and products such as condensed milk, whey, koumiss and kefir derived therefrom.

A method which affords an approximate estimation of the sugar in milk consists in adding a few drops of acetic acid and warming, filtering from the resultant curd, boiling, evaporating the clear whey to a

small bulk, again filtering, and then evaporating the filtrate to dry-ness. The residue, after drying at 130°, consists almost wholly of milk sugar and salts. The amount of the former substance present may be ascertained by igniting the weighed residue and noting the loss of weight. The amount of sugar thus obtained is always a little too high.

Estimation of Milk Sugar by Gravimetric Methods.—In esti-mating lactose in milk by Fehling's solution it is necessary to remove the proteins. This may be done by warming with a few drops of acetic acid, filtering, boiling the filtrate to coagulate the remaining proteins and again filtering. This filtrate is neutralised before adding the cop-per solution. It is better, however, to precipitate the proteins with copper sulphate.

Soxhlet's method adopted by the A. O. A. C. is as follows:

1. **Preparation of the Milk Solution.**—Dilute 25 c.c. of the milk with 400 c.c. of water and add 10 c.c. of a solution of copper sulphate of the strength given for Soxhlet's modification of Fehling's solution, page 318, add about 7.5 c.c. of a solution of potassium hydroxide of such strength that one volume of it is just sufficient to completely precipitate the copper as hydroxide from one volume of the solution of copper sulphate. Instead of a solution of potassium hydroxide of this strength 8.8 c.c. of a half-normal solution of sodium hydroxide may be used. After the addition of the alkali solution the mixture must still have an acid reaction and contain copper in solution. Fill the flask to the 500 c.c. mark, mix, and filter through a dry filter.

2. **Estimation.**—Place 50 c.c. of the mixed copper reagent in a beaker and heat to the boiling point. While boiling briskly add 100 c.c. of the lactose solution containing not more than 0.3 grm. of lactose and boil for 6 minutes. Filter immediately through asbestos and deter-mine the amount of copper reduced by one of the methods already given (page 323). Obtain the weight of lactose equivalent to the weight of copper found from the following table:

According to Soxhlet, 1 grm. milk sugar in 1 % solution reduces 322.5 c.c. of Knapp's and 214.5 c.c. of Sachsse's mercurial reagents.

TABLE FOR THE ESTIMATION OF LACTOSE.

(Soxhlet-Wein.)

Milligrams of copper.	Milligrams of lactose.	Milligrams of copper.	Milligrams of lactose.	Milligrams of copper.	Milligrams of lactose.	Milligrams of copper.	Milligrams of lactose.	Milligrams of copper.	Milligrams of lactose.
100	71.6	161	117.1	221	162.7	281	209.1	341	256.5
101	72.4	162	117.9	222	163.4	282	209.9	342	257.4
102	73.1	163	118.6	223	164.2	283	210.7	343	258.2
103	73.8	164	119.4	224	164.9	284	211.5	344	259.0
104	74.6	165	120.2	225	165.7	285	212.3	345	259.8
105	75.3	166	120.9	226	166.4	286	213.1	346	260.6
106	76.1	167	121.7	227	167.2	287	213.9	347	261.4
107	76.8	168	122.4	228	167.9	288	214.7	348	262.3
108	77.6	169	123.2	229	168.6	289	215.5	349	263.1
109	78.3	170	123.9	230	169.4	290	216.3	350	263.9
110	79.0	171	124.7	231	170.1	291	217.1	351	264.7
111	79.8	172	125.5	232	170.9	292	217.9	352	265.5
112	80.5	173	126.2	233	171.6	293	218.7	353	266.3
113	81.3	174	127.0	234	172.4	294	219.5	354	267.2
114	82.0	175	127.8	235	173.1	295	220.3	355	268.0
115	82.7	176	128.5	236	173.9	296	221.1	356	268.8
116	83.5	177	129.3	237	174.6	297	221.9	357	269.6
117	84.2	178	130.1	238	175.4	298	222.7	358	270.4
118	85.0	179	130.8	239	176.2	299	223.5	359	271.2
119	85.7	180	131.6	240	176.9	300	224.4	360	272.1
120	86.4	181	132.4	241	177.7	301	225.2	361	272.9
121	87.2	182	133.1	242	178.5	302	225.9	362	273.7
122	87.9	183	133.9	243	179.3	303	226.7	363	274.5
123	88.7	184	134.7	244	180.1	304	227.5	364	275.3
124	89.4	185	135.4	245	180.8	305	228.3	365	276.2
125	90.1	186	136.2	246	181.6	306	229.1	366	277.1
126	90.9	187	137.0	247	182.4	307	229.8	367	277.9
127	91.6	188	137.7	248	183.2	308	230.6	368	278.8
128	92.4	189	138.5	249	184.0	309	231.4	369	279.6
129	93.1	190	139.3	250	184.8	310	232.2	370	280.5
130	93.8	191	140.0	251	185.5	311	232.9	371	281.4
131	94.6	192	140.8	252	186.3	312	233.7	372	282.2
132	95.3	193	141.6	253	187.1	313	234.5	373	283.1
133	96.1	194	142.3	254	187.9	314	235.3	374	283.9
134	96.9	195	143.1	255	188.7	315	236.1	375	284.8
135	97.6	196	143.9	256	189.4	316	236.8	376	285.7
136	98.3	197	144.6	257	190.2	317	237.6	377	286.5
137	99.1	198	145.4	258	191.0	318	238.4	378	287.4
138	99.8	199	146.2	259	191.8	319	239.2	379	288.2
139	100.5	200	146.9	260	192.5	320	240.0	380	289.1
140	101.3	201	147.7	261	193.3	321	240.7	381	289.9
141	102.0	202	148.5	262	194.1	322	241.5	382	290.8
142	102.8	203	149.2	263	194.9	323	242.3	383	291.7
143	103.5	204	150.0	264	195.7	324	243.1	384	292.5
144	104.3	205	150.7	265	196.4	325	243.9	385	293.4
145	105.1	206	151.5	266	197.2	326	244.6	386	294.2
146	105.8	207	152.2	267	198.0	327	245.4	387	295.1
147	106.6	208	153.0	268	198.8	328	246.2	388	296.0
148	107.3	209	153.7	269	199.5	329	247.0	389	296.8
149	108.1	210	154.5	270	200.3	330	247.7	390	297.7
150	108.8	211	155.2	271	201.1	331	248.5	391	298.5
151	109.6	212	156.0	272	201.9	332	249.2	392	299.4
152	110.3	213	156.7	273	202.7	333	250.0	393	300.3
153	111.1	214	157.5	274	203.5	334	250.8	394	301.1
154	111.9	215	158.2	275	204.3	335	251.6	395	302.0
155	112.6	216	159.0	276	205.1	336	252.5	396	302.8
156	113.4	217	159.7	277	205.9	337	253.3	397	303.7
157	114.1	218	160.4	278	206.7	338	254.1	398	304.6
158	114.9	219	161.2	279	207.5	339	254.9	399	305.4
159	115.6	220	161.9	280	208.3	340	255.7	400	306.3
160	116.4								

Estimation of Lactose by Optical Methods.—Lactose may be estimated by observing the optical activity of its solution. To apply this method to milk, it is first necessary to prepare a clear whey free from other optically active substances. Precipitation by basic lead acetate, as has been shown by Wiley (*Amer. Chem. J.*, 1884, **6**, No. 5), does not remove completely the lævorotatory protein matters; he has proposed two alternative mercurial reagents. His method, which has been adopted by the A. O. A. C., is as follows:

a. **Acid Mercuric Nitrate.**—Dissolve mercury in double its weight of nitric acid, sp. gr. 1.42, and dilute with an equal volume of water. One c.c. of this reagent is sufficient for the quantities of milk mentioned below. Larger quantities may be used without affecting the results of polarisation.

b. **Mercuric Iodide with Acetic Acid.**—Mix 33.2 grm. of potassium iodide, 13.5 grm. of mercuric chloride, 20 c.c. of glacial acetic acid, and 640 c.c. of water.

Estimation.—The milk should be at a constant temperature, and its sp. gr. ascertained with a delicate hydrometer. When greater accuracy is required, a pycnometer is used.

The quantities of the milk measured for polarisation differ with the sp. gr. of the milk as well as with the polariscope used. The quantity to be measured in any case will be found in the following table:

Specific gravity	Volume of milk to be used	
	For polariscopes of which the sucrose normal weight is 16 19 grm.	For polariscopes of which the sucrose normal weight is 26.048 grm.
	c.c.	c.c.
1.024	60.0	64.4
1.026	59.9	64.3
1.028	59.8	64.15
1.030	59.7	64.0
1.032	59.6	63.9
1.034	59.5	63.8
1.035	59.35	63.7

Place the quantity of milk indicated in the table in a flask graduated at 102.4 c.c. for a Laurent or 102.6 c.c. for a Ventzke polariscope (Mohr c.c.). Add 1 c.c. of mercuric nitrate solution or 30 c.c. of mercuric

iodide solution (an excess of those reagents does no harm), fill to the mark, agitate, filter through a dry filter, and polarise. It is not necessary to heat before polarising. In case a 200 mm. tube is used, divide the polariscope reading by 3 when the sucrose normal weight for the instrument is 16.19 grm. or by 2 when the normal weight for the instrument is 26.048. When a 400 mm. tube is used, these divisors become 6 and 4, respectively. For the calculation of the above table the specific rotary power of lactose is taken as 52.53°, and the corresponding number for sucrose as 66.5°. The lactose normal weight to read 100° on the sugar scale for Laurent instruments is 20.496 grm.; and for Ventzke instruments, 32.975 grm. In case metric flasks are used the weights here mentioned must be reduced accordingly.

In the foregoing, allowance (2.6 c.c.) is made for the volume of the precipitate. To eliminate the errors which may arise in this way Wiley and Ewell have applied Scheibler's method of double dilution to the determination of lactose. The following is a summary of the process: For polarimeters adapted to the normal weight of 26.048 sucrose, 65.82 grm. of milk are placed in a 100 c.c. flask clarified with 10 c.c. of the acid mercuric nitrate, the volume made up to the 100 mark, the liquid well shaken, filtered, and the rotation determined. A similar quantity of milk is put into a 200 c.c. flask, acid mercuric nitrate added (it may be necessary to use more than 10 c.c. in this case), the liquid made up to the 200 c.c. mark, shaken, and the rotation determined in a 100 mm. tube. The true polarimetric reading is obtained by dividing the product of the two readings by their difference.

H. D. Richmond (Dairy Analysis) corrects for the sp. gr. and percentage of fat of the milk as follows: To 50 c.c. of milk a quantity of water is added equal in c.c. to the sum of—

a. The degrees of gravity divided by 20.

b. The percentage of fat divided by 1.8.

c. A factor to convert scale readings into percentages of anhydrous sugar. This is 5.43 c.c. if the scale is in angular degrees and a 200 mm. tube is used.

1.5 c.c. of Wiley's acid mercuric nitrate solution is then added and after violent shaking the solution is filtered through a dry filter.

Richmond, gives the following example: A milk has a sp. gr. of 1.032 and 3.60% of fat. (a) is 32.0/20 = 1.6 c.c., (b) = 3.6/1.8, (c) = 5.43 c.c. The water added is therefore, 1.6 + 2.0 + 5.43 = 9.03 c.c.

In the case of human milk, clarification is more difficult and a turbid

filtrate is obtained with ordinary precipitating agents. Thebault (*J. Pharm.* (6), 4, 5) uses a solution of picric acid 10 grm. in 1000 c.c. and acetic acid 25 c.c. in 1000 c.c.

Estimation of Sugars in Condensed Milk.—To estimate lactose in condensed milk, the proteins must be removed by precipitation and the reducing sugar in the whey determined. The cane sugar present may be approximately estimated by difference; that is, subtracting the sum of the other ingredients from the total solids. The direct methods are all based on the removal of the proteins and determination of the cupric reducing or optical rotatory power before and after inversion. This must be effected by a mild agent, such as citric acid or invertase since mineral acids will also hydrolyse the lactose.

Stokes and Bodner (*Analyst*, 1885, 10) coagulate the milk by the addition of 1% of citric acid without heating, dilute, filter and esti-mate the reducing power of the clear filtrate. To another portion of the filtrate a further 1% of citric acid is added and the solution boiled for 10 minutes according to the authors or better, for at least 30 minutes, according to Watts and Tempany (*Analyst*, 1905, 30, 119).

The solution is cooled, neutralised and the reducing power again determined. The increase is due to the invert sugar formed from the sucrose.

Leffmann and Beam use invertase for inversion. The proteins are precipitated by mercuric nitrate and the clear whey polarised. In a portion of the filtrate, the acid is carefully neutralised, a drop of acetic acid is added and a small quantity of invertase along with a few drops of an antiseptic. The whole is incubated at 35° to 40° for 24 hours. After the action, the proteins are precipitated by alumina cream and the liquid made to known volume and again polarised. The difference between the two readings is calculated as sucrose.

Bigelow and McElroy (*J. Amer. Chem. Soc.*, 1893, 15) propose the following routine method for the determination of the sugars, including invert sugar, in condensed milk. The solutions used are:

Acid Mercuric Iodide.—See page 368. *Alumina Cream.*—See page 309.

The entire contents of a can are transferred to a porcelain dish and thoroughly mixed. A number of portions about 25 grm. each are weighed carefully in 100 c.c. flasks. Water is added to two of the por-tions and the solutions boiled. The flasks are cooled, clarified by means of a small amount of each of the above solutions made up to the mark,

shaken, filtered, and the polarimetric reading noted. Other weighed portions are heated in the water-bath to 55°, one-half of a cake of compressed yeast added to each flask, and the temperature maintained at 55° for 5 hours. The solutions are then clarified as before, cooled to room temperature, made up to 100 c.c., mixed, filtered, and the polarimetric reading taken. The amount of cane sugar is determined by the formula on page 313. Correction for the volume of precipitated solids may be made by the double-dilution method. The total reducing sugar is estimated by one of the reducing methods on one of the weighed portions of the original material, and if the sum of it and the amount of cane sugar determined by the inversion method is equal to that obtained by the direct reading of both sugars before inversion, no invert sugar is present. If the amount of reducing sugar seems too great, the milk sugar must be redetermined as follows: 250 grm. of the sample are dissolved in water, the solution boiled, cooled to 80°, a solution of about 4 grm. of glacial phosphoric acid added, the mixture kept at 80° for a few minutes, then cooled to room temperature, made up to a definite volume, mixed, and filtered. It may be assumed that the precipitate produced by the phosphoric acid is equal in volume to that produced by the acid mercuric iodide. Potassium iodide is then added in amount not quite sufficient to neutralise the acid, and sufficient water to make up for the solids precipitated by the acid. The mixture is then filtered and the filtrate measured in portions of 100 c.c. into 200 c.c. flasks. A solution containing 20 mg. of potassium fluoride and half a cake of compressed yeast is added to each flask, and the mixture allowed to stand for 10 days at a temperature of from 25° to 30°. The invert sugar and cane sugar are fermented and removed while the milk sugar is unaffected. The flasks are filled to the mark, shaken, and the milk sugar determined by either reduction or the polariscope. The amount of copper reduced by the milk sugar and invert sugar, less the equivalent of milk sugar remaining after fermentation, is due to invert sugar.

C. B. Cochran (*J. Amer. Chem. Soc.*, 1907, 29, 555–556) makes use of Wiley's acid mercuric-nitrate solution to invert sucrose in the analysis of sweetened condensed milk. He finds this inverts sucrose only very slowly at temperatures below 15°. 50 c.c. of the solution to be inverted (containing 3 c.c. of mercuric solution per 100 c.c.) are polarised as soon as possible after the solution has been mixed at 15° and then heated in boiling water for 7 minutes and again polarised.

The sucrose content in the case of normal solutions is given by the formula:

$$\text{Sucrose} = \frac{100\,D}{132.68 - 0.5\,t}$$ where D is the difference in polarisation

before and after inversion and t = temperature above 20° C. To detect sucrose in condensed milk or milk sugar, Leffmann applies the sesame oil test. 1 c.c. of sesame-oil, 1 c.c. of concentrated hydrochloric acid and 0.5 grm. of the sample are shaken together. The characteristic crimson colouration will be formed within half an hour.

This test has been found to be satisfactory and is better than that given in United States Pharmacopœia which depends on carbonisation of the sucrose by strong sulphuric acid. A rapid test for sucrose in milk and cream consists in boiling a mixture of 15 c.c. of milk, 0.1 grm. of resorcinol and 1 c.c. of concentrated hydrochloric acid. Sucrose gives a fine red colouration, pure milk remains almost unchanged.

MONOSACCHARIDES.

Of commercial importance are the hexoses $C_6H_{12}O_6$, more generally termed glucoses, and the pentoses $C_5H_{10}O_5$.

Glucoses.—Their generic and specific characters are contained in the table on page 287. Since they are very closely related in structure, differing indeed, with the exception of lævulose, only in the space arrangement of the groups in their molecule, their chemical properties are very similar. As a class they are (1) not susceptible of inversion; (2) are readily and directly fermented by yeast; (3) are decomposed by alkalies; (4) are readily oxidised by alkaline solutions of copper.

Dextrose.—d-Glucose.

This is produced from various polysaccharides and glucosides by hydrolysis with acids or enzymes and also from cellulose materials. It is found ready formed in various fruits, the proportion in grapes being as high as 15%. It exists in two isomeric modifications which are mutually transformed into one another in solution. The a-isomeride, which crystallises from aqueous solutions as a hydrate, forms tabular crystals. It is obtained in transparent prisms by crystallisation from hot methyl alcohol, melting at 146°. It is less soluble than sucrose, requiring 1 1/3 times its own weight of cold water. It has the value $[a]_D = 52.7°$, $[a]_J = 58.5°$ for the anhydride and exhibits mutarotation

(see page 315). The change in rotation with the concentration (c) may be calculated from the formula (Tollens, *Ber.*, 1884, **17**, 2234):

$$[a]_D = +52.5 + 0.018796c + 0.00051683c^2.$$

The rotation is constant from o to 100° C.

Other properties of dextrose and methods of estimating it have been already described.

Characteristic of dextrose (and glucuronic acid) is the formation of saccharic acid on oxidation. 5 grm. of the sugar are heated at the temperature of the water-bath with 30 c.c. nitric acid (sp. gr. 1.15) to a thick syrup. This is taken up with water and again evaporated to remove excess of acid, neutralised with potassium carbonate and a few drops of acetic acid added, when the potassium saccharate crystallises out on standing.

Characteristic also is the very insoluble phenylosazone, m. p. 205–210°. Tutin gives this as 217° on recrystallisation from pyridine (*Proc. Chem. Soc.*, 1907, **23**, 50), but the older value is the correct one (Fischer, *Ber.* 1908, **41**, 73).

Dextrose diphenylhydrazone, m. p. 161°, and the methylphenyl-hydrazone, m. p. 130°, may be used to identify or detect dextrose, particularly in presence of pentoses.

Lævulose.—d-Fructose.—Fruit Sugar.

Lævulose differs from dextrose in containing a ketonic grouping. It occurs together with dextrose in honey and many fruits and is produced together with dextrose on hydrolysis of sucrose or with dextrose and other sugars on hydrolysis of some polysaccharides. Inulin, a polysaccharide found in dahlia tubers, yields lævulose alone when hydrolysed. No glucoside containing lævulose has been as yet isolated. The principal physical and chemical properties of lævulose are contained in the table on page 287. Its behaviour is very similar to that of dextrose, but it is not readily crystallisable. It yields glycollic acid when oxidised with bromine water and not gluconic acid.

Lævulose has a value $[a]_D^{15} = -93.8°$, which value decreases 0.6385° for each rise of 1° C. in the temperature. The rotation at 87.2° C. is −52.7°; that is equal, but opposite, to that of dextrose at the same temperature.

This change in the optical activity of lævulose affords a means of estimating it in presence of other sugars. The solution is examined in a jacketed polarimeter tube provided with a thermometer and the

rotation noted at two temperatures as far apart as possible. It is advisable to use fairly strong solutions. To calculate the number of grm. of lævulose in 100 c.c. of solution, the difference between the two temperatures is multiplied by 1.277 and this product divided into 100 times the alteration in rotation measured in circular degrees in a two dcm. tube by sodium light. In view of the fact that the rotatory power of other sugars likewise differs with the temperature, this method can hardly claim any great accuracy.

The respective reducing actions of dextrose and lævulose on Fehling's copper solution are usually assumed to be identical. According to Soxhlet, however, the reducing action of the former is sensibly greater than that of the latter. Allihn states that the reducing action of dextrose and lævulose are identical if care be taken to continue the boiling of the solution for half an hour.

Lævulose, on account of its ketonic nature, reduces alkaline copper solutions more rapidly than do other sugars and at a lower temperature and this property may be made use of for its estimation. The subject has of late been fully investigated by J. Pieraerts (*Bull. Assoc. Chim. Sucr. et. Dist.*, 1908, 25, 830), who has made comparative trials of a number of reagents. Excellent results were obtained with a cupro-glycocoll solution (6 grm. of cupric hydroxide, 12 grm. of glycocoll and 50 grm. of potassium carbonate dissolved in water and made up to 1000 c.c.) which is reduced by lævulose at normal temperature in 12 hours, but totally unaffected by other hexose or pentose sugars in 24 hours. For the determination of lævulose in commercial preparations the following general method is prescribed. 20 to 25 grm. of material are dissolved in 150 to 200 c.c. of cold water, clarified with lead basic acetate and excess of lead removed by saturated sodium-sulphate solution. After half an hour, the solution is filtered and diluted so as to contain 5% reducing sugar and tested for lævulose as above.

Other suitable reagents are: Alkaline cupric hydroxide (100 grm. of potassium carbonate, 50 or 75 grms. of potassium hydrogen carbonate and 6 grm. of cupric hydroxide in 1000 c.c.). With this lævulose may be detected with certainty even when much pentose is present. The action is continued for 3 hours at the ordinary temperature or, in the absence of pentose, for 1 hour at 30°.

1 grm. lævulose in 1% solution reduces 508.5 c.c. of Knapp's and 449.5 c.c. of Sachsse's mercury reagents. The reducing action

on Knapp's solution is about the same as that of dextrose, but dextrose has a considerably weaker reducing action on Sachsse's solution, equal amounts of dextrose and lævulóse reducing 100 c.c. and 148.6 c.c. respectively.

According to Neuberg, the methyl-phenyl-osazone of lævulose is very characteristic. It forms long yellow needles, m. p. 158 to 160° C.

The following formula for the estimation of lævulose and dextrose in mixtures is given by Pellet (*Bull. Assoc. Chin. Sucr. Dist.*, 1907, **25**, 125–127). Let 100 grm. of the mixture contain x grm. of dextrose and y grm. of lævulose of which p and p' are the polarising values compared with sucrose and r, r' the reducing powers compared with invert sugar. P (the specific rotation of the mixture) will equal $px +$ $p'y$; R (the quantity of the reducing sugars) will equal $rx + r'y$. Now $p = 0.793$, $p' = 1.356$, $r = 0.960$, $r' = 1.04$ whence $P = 0.793x - 1.356y$, $R = 0.96x + 1.04y$.

$$\text{Therefore } x = \frac{1.356R + 1.04P}{2.126}, \quad y = \frac{0.793R - 0.960P}{2.126}$$

Invert Sugar.—Invert sugar exists largely in honey, molasses, and many fruits. It is a mixture of equivalent proportions of dextrose and lævulose, produced by the action of heat, some enzymes, acids, salts or other agents on cane sugar and some of its isomers. The conditions most favourable for its formation have already been described.

Invert sugar is an uncrystallisable syrup having a sweeter taste than cane sugar. In its chemical reactions and optical properties it behaves strictly as a mixture of dextrose and lævulose. Invert sugar is now made largely for brewers' use, being sold under the names of "invert" or "inverse sugar," "saccharum," "malt-saccharum," and other trade names. Starch-sugar and cane sugar are often added. The analysis of such products may be effected in the same manner as that of honey, but it is generally sufficient to estimate the sugar by Fehling's solution before and after inversion. These estimations give the data for calculating the cane or uninverted sugar and the total invert sugar without distinguishing between the dextrose and lævulose. The small quantity of sucrose which is generally present cannot be estimated accurately by double polarisation.

Analyses of Invert Sugar (Typical).—From Moritz and Morris'
"Text-book of the Science of Brewing."

	Good.	Inferior.
Invert sugar,	75.23	60.53
Cane sugar,	0.95	8.56
Ash,	1.16	5.53
Proteins,	0.78	1.89
Water,	19.23	13.77
Other matters,	2.65	9.72
	100.00	100.00

Galactose.—Galactose is formed together with dextrose by the hydrolysis of milk sugar; it occurs in some glucosides, most gums and many plant products. It is much less sweet than sucrose, yields dulcitol on reduction and galactonic acid when oxidised with bromine water.

It has a value of $[a]_D = 81.27°$ for a 10% solution at 15° and the rotation at temperature t, and concentration c may be caclulated from the formula:

$$[a]_D = 83.137 + 0.199c - (0.276 - 0.0025c)t$$

It exhibits mutarotation. Other physical data will be found on page 287

Characteristic is the a-methylphenylhydrazone which forms colourless needles, m. p. 180°, and is sparingly soluble in water and absolute alcohol. It may be separated from dextrose by such yeasts as *S. apiculatus and S. Ludwigii*, which ferment dextrose, but not galactose (see page 287). A fermentation test with one of these yeasts affords a very delicate means of detecting the presence of small quantities of in a sample of galactose. Commercial galactose invariably contains a small percentum of dextrose.

Characteristic of galactose and also of lactose is the formation of mucic acid on oxidation with nitric acid.

For the preparation of mucic acid the sugar should be slowly evaporated on the water-bath with about 4 times its weight of nitric acid of 1.27 sp. gr. or 10 times its weight of acid of 1.15 sp. gr. until a thick syrup is formed. This is diluted with a little water and allowed to stand for some hours. Mucic and oxalic acid will crystallise

out and may be separated by warm alcohol in which only the oxalic acid dissolves.

Tollens (*Annalen*, 1885, 227, 223) applies this method quantitatively as follows: 5 grm. of the dry sugar are placed in a beaker 6 cm. in diameter with 50 c.c. of nitric acid, sp. gr 1.15, and evaporated at the heat of the water-bath to 1/3 of its volume. When cold 0.5 grm. of pure mucic acid and 100 c.c. of water are added. After 1 or, better, 2 days' standing with occasional stirring the crystallised solid is collected on a weighed filter, washed twice with 5 c.c. of cold water, dried at 100° and weighed. After subtraction of the 0.5 grm. of mucic acid added to facilitàte crystallisation, 77.4 parts of mucic acid correspond to 100 parts of galactose.

Should impurities, such as cellulose or calcium salts, remain after washing, the precipitate and filter-paper are warmed with a solution of ammonium carbonate, filtered and the filtrate evaporated nearly to dryness in a dish. The mucic acid is precipitated on the addition of nitric acid and may be collected and weighed.

This method has proved useful in the estimation of the galactose-yielding groups in complex carbohydrates; in presence of large quantities of foreign, organic matter, however, the crystallisation of mucic acid may be hindered or altogether prevented.

It has been adopted by the A. O. A. C. for the estimation of galactan the amount of which is obtained by multiplying the weight of mucic acid by 1.197.

Commercial Glucose—Starch-sugar.—Several partially or fully converted starch-sugars of which dextrose is the leading constituent are sold under the name of glucose, starch-sugar and a variety of other more fanciful appelations. Commercial glucoses are very largely used as substitutes for other carbohydrates, *e. g.*, for malt in brewing, in honey and for the manufacture of factitious wine.

Starch-glucose occurs in commerce in several forms, ranging from the condition of pure anhydrous dextrose, through inferior kinds of solid sugar, to the condition of a thick, syrupy liquid resembling glycerin, which contains a large proportion of dextrin.

In America, the term "glucose" is restricted to the syrupy preparations, the solid products being distinguished as "grape sugar." The following grades are recognised:

Liquid Varieties.—Glucose, mixing glucose, mixing syrup, corn syrup, jelly glucose and confectioners' crystal glucose.

Solid Varieties.—Solid grape sugar, clipped grape sugar, granulated grape sugar, powdered grape sugar, confectioners' grape sugar, brewers' grape sugar.

The United States standard of purity states that glucose, mixing glucose or confectioners' glucose has a sp. gr. at 100° F. of from 41° B (21 % water) to 45° B (14 % water) and contains not more than 1 % ash on a basis of 41° B.

Commercial starch-glucose is produced by the action of dilute acid on starch or starchy matter or occasionally woody fibre. In America it appears to be wholly made from maize starch, and is often termed "corn syrup," but in Europe rice and potato starches are frequently used.

As a rule, in the United States, hydrochloric acid is the converting agent, the proportion employed ranging in practice from 1 to 3 % according to the kind of product desired and the details of the subsequent manipulation. The starch, or amylaceous substance, is either boiled with the acid and water in an open tank or heated with it in strong copper cylinders under high pressure. If the first method be adopted and the process arrested as soon as a cold sample of the liquid ceases to give a blue colour with iodine, the product contains a large proportion of dextrin; but if high pressure be employed · and the action pushed further, dextrose is the chief product. In either mode of operating, maltose and, very commonly, other products are formed in addition to dextrose and dextrin. The acid is next neutralised, the liquid decolourised, if necessary, by animal charcoal and evaporated *in vacuo* till it acquires a sp. gr. of 1,400 to 1,420.

When sulphuric acid is used as the converting agent the product retains a considerable quantity of dissolved calcium sulphate. Oxalic acid is also sometimes used. As a result of the method of manufacture, inferior qualties of glucose may contain sulphurous or sulphuric acid, calcium sulphate or chlorides, arsenic and lead compounds. Arsenic may be detected by Reinsch's test or by the methods referred to under "Malt"; for lead see p. 569. The mineral matter may be determined by the weight and composition of the ash, which should not in a good product exceed 1 % and should be almost wholly free from iron if the glucose is to be used for brewing. Sometimes the calcium sulphate is removed by treating the concentrated solution with barium oxalate. The amount of free acid is estimated by titration with standard alkali and phenolphthalein; many specimens possess normally a slightly acid

reaction, probably due to acid phosphates. Water may be determined by one of the methods given on page 343, a high temperature being carefully avoided. Nitrogenous matter is conveniently determined by the Kjeldahl process or by ignition with soda-lime.

Most commercial starch-sugars contain, in addition to dextrose and dextrin, maltose and a notable percentage of unfermentable carbohydrates, apparently produced by overtreatment with acid. The term gallisin is applied to these.

The majority of the published analyses of glucose products have failed to take all these products into account or are based on faulty methods of analysis. In consequence,much confusion exists on this particular subject.

Gallisin as hitherto obtained is not a definite compound and it appears advisable only to retain the term as synonymous with unfermentable matter. The whole question of the structure of starch, the nature of the various dextrins and of isomaltose still remains a vexed question in carbohydrate chemistry and the utmost confusion exists as regards the subject. (The reader is referred to Ling's article on Starch in Sykes' " Text-book of Brewing," edition 1907.)

The following analyses of commercial glucoses, quoted by W. G. Valentin (*Jour. Soc. Arts*, **24**, 404) are amongst the most complete and probably most reliable hitherto published:

	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.
Dextrose	80.00	58.85	67.44	63.42	61.46
Maltose	none	14.11	10.96	13.50	13.20
Dextrin	none	1.70	none	none	none
Unfermentable carbohydrates with a little protein matter	8.20	9.38	4.30	8.40	8.60
Mineral matter	1.30	1.40	1.60	1.50	1.60
Water	10.50	14.56	15.70	13.18	15.20
	100.00	100.00	100.00	100.00	100.06
Total solid matter	89.50	85.44	84.30	86.82	84.80
Matter of use to the brewer	80.00	74.66	78.40	76.92	74.60

No. 1 was somewhat brown, very hard, and of English manufacture. No. 2 was pale straw-coloured, softish, French. No. 3, whitish, some-

what hard, English. No. 4, whitish, somewhat hard German. No. 5, white, somewhat hard, German.

The following analyses are by I. Steiner (*Dingler's Polyt. Jour.*, 233, 262):

	No. 1.	No. 2.	No. 3.	No. 4.
Dextrose................................	45.40	26.50	76.00	....
Maltose	28.00	40.30	5.00	42.60
Dextrin................................	9.30	15.90	....	39.80
Unfermentable carbohydrates...	1.50	7.00	5.30	8.90
Proteins..............................	traces	1'.80	.20	....
Free acid (as H_2SO_4)	.08	.03	.05	....
Mineral matter.......................	.30	2.50	.40	1.10
Water................................	15.50	6.00	13.30	7.60
	100.08	100.03	100.25	100.00
Total solid matter..............	84.42	93.97	86.65	92.40
Matter of use of the brewer.....	82.70	82.70	81.00	82.40

No. 1 was of German origin, white and soft. The other samples were English, and made from maize without previous separation of the starch.

These analyses are unusually elaborate, and for commercial purposes there is no occasion to enter so much into detail. Many analysts limit their statements to the proportions of water, ash, dextrin, and glucose, ignoring the maltose altogether. This practice is very objectionable, as, in an analysis so stated not only is the maltose classed as dextrose, but the amount of dextrin is also seriously in error. Nevertheless, the cupric reducing power of the sample is a character of considerable value for the commercial classification of a glucose or for assaying a sample during the process of conversion, provided its true meaning be not misinterpreted. Taken together with the specific rotatory power of a sample, and the percentage of ash and water, it often affords ample information for commercial purposes.

The following data allow the relative proportions of dextrose, maltose and dextrin in a sample of glucose to be deduced. The total solids are ascertained from the solution gravity or by carefully drying the sample, and the residue is ignited to ascertain the ash. The difference gives the organic solids (O). The reducing power (K) and the specific rotatory power (S) of the sample are further determined.

Then if m be the percentage of maltose, g that of dextrose and d that of dextrin in the sample

$$m = S - \frac{52.7K + 198(O - K)}{100} \div 0.313$$

$$g = K - 0.62m$$

$$d = O - (g + m)$$

Defects of the method are that K and S have to be ascertained very accurately and that the presence of unfermentable bodies, such as gallisin, which exert a reducing action, is ignored.

Wiley bases a process on the assumption that dextrose and maltose are oxidised to optically inactive products when heated with excess of an alkaline solution of mercuric cyanide and that dextrin is unaffected. The following is the mode of operation adopted by Wiley (*Chem. News*, 1882, **46**, 175):

a. The cupric reducing power of the sample is ascertained in the usual way by Fehling's solution.

b. The specific rotatory power is ascertained by polarising a 10% solution (previously heated to boiling) in the ordinary manner.

c. 10 c.c. of the solution employed for *b* (= 1 grm. of the original sample) is treated with an excess of an alkaline solution of mercuric cyanide,[1] and the mixture boiled for 2 or 3 minutes. It is then cooled and slightly acidified with hydrochloric acid, which destroys the reddish-brown colour possessed by the alkaline liquid. The solution is then diluted to 50 c.c., and the rotation observed in a tube 4 decimetres in length. The angular rotation observed will be due simply to the dextrin, the percentage of which in the sample may be calculated by the following formula:[2]

The percentages of dextrose and maltose may be deduced from the reducing power of the sample, or from the difference between the

[1] The mercuric solution is prepared by dissolving about 120 grm. of mercuric cyanide and the same quantity of sodium hydroxide in 1000 c.c. and filtering the liquid through asbestos. 20 c.c. of this solution should be employed for samples having K less than 65 per cent., and 25 c.c. when the reducing power is greater than this. In all cases care must be taken to use a slight excess of the mercuric solution, which may be ascertained by holding a piece of filter-paper with a drop of the solution on it over fuming hydrochloric acid, and then over ammonia or hydrogen sulphide water, when a dark stain, due to mercuric sulphide, will appear on the paper.

[2] If the directions in the text are adhered to, and a sample shows an angular rotation of 3.2° with a tube 4 decimetres in length, then the calculation will be:

$$\frac{3.2 \times 1000 \times 50}{198 \times 40 \times 1} = 20.20\% \text{ of dextrin.}$$

$$\frac{\text{Circular rotation} \times 1000 \times \text{volume in c.c. of solution polarized}}{198 \times \text{length of tube in centimetres} \times \text{weight of sample in solution employed for mercury treatment.}} = \text{Percentage of dextrin.}$$

specific rotatory power before (S) and after (s) the treatment with the alkaline mercuric solution. Using the same symbols as before, with the addition of u for the unknown and presumed inactive organic matter, the following equations result:

$$O = g + m + d + u; \quad K = 1.00\, g + 0.62m.$$
$$S = 0.527g + 1.392m + 1.98d; \quad s = 1.98d.$$

From these data:

$$S - s = 0.527g + 1.392m; \quad \text{and} \quad 0.527K = 0.527g + 0.32674m; \quad \text{whence}$$
$$1.06526m = S - s - 0.527K; \quad m = \frac{S - s - 0.527K}{1.06526}$$

The proportions of dextrose, dextrin, and inactive carbohydrates are deduced by means which are evident.

In Wiley's process it is assumed that the indefinite carbohydrates have no optical activity and no reducing action on Fehling's solution. Both these assumptions are probably incorrect, in addition to which it has not been definitely proved that boiling with an alkaline solution of mercuric cyanide wholly destroys the optical activity of maltose and dextrose, while leaving that of dextrin unchanged. Nor has the action of the mercuric solution on the indefinite carbohydrates been ascertained with certainty, though they may be presumed to react like maltose, since "gallisin" is stated to reduce Knapp's solution.

It is manifestly impossible to determine with absolute accuracy the amount of commercial glucose added as an adulterant by reason of the differing amounts of dextrose, maltose and dextrin present in commercial glucose.

When the amount of invert sugar present is very small an approximate result is obtained on the assumption that commercial glucose polarises $+175°$ V.

The formula $g = (a - s)100/175$ is used when $g = \%$ of commercial glucose, $a = $ direct polarisation, $s = \%$ of sucrose.

In substances which consist largely of invert sugar much more accurate results are attained by polarising at $87°$ in a water-jacketed tube an inverted half-normal solution of the sample (13 grm.) prepared as described elsewhere with the following exceptions: After inversion, cool, add a few drops of phenolphthalein and enough sodium hydroxide to neutralize; discharge the pink with a few drops of dilute hydrochloric acid, add from 5 to 10 c.c. of alumina cream, and make up to the mark and filter. Multiply by 2 the reading at $87°$ in the

200 mm. tube; multiply this result by 100 and divide by the factor 163 to express the glucose in terms of glucose polarising 175° Ventzke.

HONEY.

Ordinary honey is a saccharine substance collected and stored by a particular species of bee (*Apis mellifica*).

Honey is essentially a concentrated aqueous solution of certain sugars, dextrose and lævulose being the chief constituents. In some cases small amounts of sucrose are present and also a sensible quantity of the alcohol mannitol. Honey, particularly when of coniferous origin, also sometimes contains some quantity of a carbohydrate intermediate between starch and sugar which is precipitated by strong alcohol. These are termed *honeydew* honeys in America. The other constituents are water, formic and other organic acids and other small proportions of mineral and flavouring matters, wax and débris in the form of pollen, insects' wings, etc. Not infrequently alkaloidal and bitter principles derived from the pollen are also met with.

Genuine honey should contain not more than 8% sucrose, 25% water and 0.25% ash. It should contain dextrose and lævulose in about equal proportions and be lævorotatory. Honey of coniferous origin, however, gives genuine dextrorotatory samples.

Although the figures representing the other constituents show considerable range, the great majority of samples of honey are of a reemarkably constant character, the glucoses ranging from 70 to 80%, the water from 17 to 20%, and the ash from 0.10 to 0.25%. In normal honey the dextrose and lævulose are present in approximately equal proportions, but if the honey has crystallised in the comb the runnings therefrom will be deficient in dextrose, and hence will be strongly lævorotatory. It is held by experienced bee-keepers that all genuine honey will eventually crystallise, and hence that honey warranted to remain syrupy is probably adulterated.

The composition and analysis of American honeys has been recently very fully studied by C. A. Browne (*Bulletin* 110), who has investigated the general composition of honeys with particular reference to the effects of different floral nectars. He gives the following average analyses of 99 samples. The table also includes the average of recent analyses of European honeys by König (*Chem. Nahrungs- und Genuss-*

mittel, 3d edition)—138 samples—and by Lehman and Stadlinger—17 samples (*Zeit. Nahr. u Genussm.*, 1907, **13,** 397).

	Browne			König			Lehman and Stadlinger
	Lævorotatory honeys 92 samples	Dextrorotatory honeys 7 samples	American honeys average 99 samples	Minimum	Maximum	Average	
Water	17.7	16.1	17.6	10.0	33.6	20.6	19.3
Invert sugar	75.	67.	74.4	54.	91.58	73.1	73.45
Sucrose	1.9	3.0	2.0	—	12.9	1.76	3.1
Ash	0.18	0.81	0.23	0.02	0.68	0.25	0.09
Undetermined ...	3.7	3.4	3.7				4.07
Free acid as formic	0.08	0.12	0.09				0.07

The following figures give the average composition of genuine (Canadian?) honey (Canadian Dept. In. Rev.; Bul., 47):

Sucrose (by Clerget),	0.5 to 7.64 per cent.
Dextrose and lævulose,	66.37 to 78.80 per cent.
Water,	12.0 to 33.00 per cent.
Ash,	0.03 to 0.50 per cent.

Analysis of Commercial Honey.—The common adulterants of honey are starch-sugar, invert sugar, cane sugar, and molasses.

The proportion of *water* in honey may be determined as in molasses (page 356), or by the method of Wiley, described on page 353. A useful check on the result is obtained by calculating the solids from the density of a 20% solution of the sample, as described on page 290.

The **ash** of genuine honey is usually very trifling in amount. If in excess of 0.3%, it should be tested for *calcium sulphate*, the presence of which, in notable quantity, is an almost certain indication of adulteration by starch glucose or invert sugar. Sulphates may also be detected by the direct addition of barium chloride to the aqueous solution of the sample. A high ash containing a notable proportion of *chlorides* points to a probable adulteration with molasses.

The insoluble matter of honey may be ascertained as in sugar. It usually consists of wax, pollen and some minor organised materials, and should be carefully examined under the miscrocope. **Starch,** which is not a normal constituent of honey, will be readily recognised in

the residue by its reaction with iodine, and, if present in quantity, points to an adulteration of the sample with flour or other farinaceous substance, the exact nature of which will be indicated by its microscopic appearance.

Gelatin, if present, will be left undissolved on treating the sample with spirit, and will be recognised by its odour on ignition, and the reaction of its aqueous solution with tannin.

Dextrin, which is not found in genuine honey, but is a constituent of commercial starch-sugar, may be detected by diluting the honey with an equal measure of water, and gradually adding strong spirit, stirring constantly until a permanent turbidity is produced. In samples adulterated with starch-sugar a heavy gummy deposit will soon form, but with genuine honey only a slight milkiness is produced.

Saccharine additions to honey can only be detected by a careful examination of the action of the sample on polarised light, and its behaviour with Fehling's and other reducible solutions. The following table shows the specific rotatory power and cupric reducing power of mixtures of cane and invert sugar, containing 82% of the solid and 18% of water, and of average starch-sugar syrup, as compared with genuine honey. The table also shows the changes produced in solutions of the above saccharine matters by the action of invertase (page 314), by prolonged heating with dilute acid (page 296), and by fermentation with yeast (page 298):

	Cane sugar 82%, water 18%	Invert sugar 82%, water 18%	Average starch-sugar, syrup	Genuine honey
Specific Rotatory Power for Sodium Ray:				
Original substance,	+54.5	—18.9 at 15°	+92 to 100	+2 to —3
After treatment with invertase,	—19.9 at 15°	—18.9 at 15°	little altered	little altered
After prolonged heating with dilute acid,	—19.9 at 15°	—18.9 at 15°	+45	little altered
After fermentation with yeast,	inactive	inactive {	very notably dextrorotatory	} 0 to+4
Cupric Reducing Power:				
Original substance,	.0	82	53	61 to 82
After treatment with invertase,	86.3	82	little altered	little altered
After prolonged heating with dilute acid,	86.3	82	82	little altered
After fermentation with yeast,	.0	0	very notable	0 to 2

Invert Sugar.—According to the table, there is a sensible difference between the rotation of *invert sugar* and genuine honey, but unfortunately this distinction does not always hold good, for if the honey has crystallised in the comb some of the dextrose is apt to re-

main there, and the honey drained therefrom will contain excess of
lævulose, and be more strongly lævorotatory than is indicated by the
figures in the table. Unless, therefore, the ash be excessive, or happen
to contain calcium sulphate, the positive recognition of added invert
sugar by such means is impossible.

The artificial honey made by Herzfeld's method (*Zeits. Ver. d.
Zucker Ind.*, 31, 1988), which consists in heating 1,000 grm. of refined
sugar with 300 c.c. of water and 1.1 grm. of tartaric acid to boiling
for 30 to 45 minutes, has a rich golden-yellow colour and a mild, pleasant
flavour. On analysis, but for a deficiency in ash, it gives values agreeing
very closely with those recorded for pure honeys.

To detect the presence of added invert sugar it is necessary to have
resource to colorimetric tests. C. A. Browne (*Bulletin* 110) uses ani-
line acetate which gives a red or pink tint with furfuraldehyde pro-
duced at the high temperature of inversion by the Herzfeld and other
processes, but no colouration with pure honey. The reagent is freshly
prepared each time before use by shaking 5 c.c. aniline with 5 c.c.
water and enough glacial acetic acid (2 c.c.) to just clear the emulsion.
1 to 2 c.c. of the reagent are allowed to flow down the walls of a test-
tube onto 5 c.c. of a solution of the honey in an equal weight of
water. If after gentle shaking a red ring forms below the aniline
layer and gradually spreads to the whole solution, invert sugar is
present.

Fiehe (*Chem. Zeit.*, 1900, 32, 1045) describes the following method·
The ethereal extract of artificial honeys on evaporation gives an intense
red colouration with resorcinol hydrochloric acid (1 part of resorcinol
and 100 parts of hydrochloric acid, sp. gr. 1.19). This is due to decom-
position products of lævulose formed by heating invert sugar with
acids during the manufacture. Natural honey which has not been
heated with acid never gives the reaction, nor does glucose, galactose,
milk-sugar, or maltose. The ammoniacal silver test of Ley (*Pharm.
Zeit.*, 1902, 47, 603) consists in heating a solution of the honey with a
small quantity of silver oxide dissolved in ammonia. Clear honeys
give a brownish colour and leave a yellowish-green on the surface of
the glass. Honey substitutes appear a dirty brown or black and give
no greenish after-hue.

Cane Sugar.—Any considerable proportion of *cane sugar* in
honey would be indicated by the strong dextro-rotation of the sample,
changed to left-handed rotation on treatment with invertase or dilute

acid. The proportion of cane sugar can be estimated from the extent of the *change* in the rotatory and reducing power of the sample caused by treatment with invertase, or, in the absence of starch-sugar, by inversion with dilute hydrochloric acid, as on page 313. As already stated, a small percentage of sucrose appears sometimes as a constituent of genuine honey. For calculating the percentage of sucrose, Lehmann and Stadlinger *Zeit. Nahr. u. Genussm.*, 1907, **13**, 397-413) give the formula:

Per cent. sucrose $=[a]_D \times 1.1448$ in which $[a]_D$ is the difference in the specific rotatory power before and after inversion.

Starch-sugar is still more dextrorotatory than cane sugar to commence with, the optical activity falling to about one-half by prolonged treatment with acid, while the products left after fermentation are still notably dextrorotatory. In the absence of added cane and invert sugar, an approximate estimation of the proportion of starch syrup in honey may be made by reckoning 1% of the adulterant for every degree of dextrorotatory power possessed by the original sample. Of course, it must not be forgotten that a dextrorotation of a few degrees degrees is observable in genuine coniferous honey dew honey.

E. Beckmann (*Zeit. anal. Chem.*, 1896, 263) tests honey for the addition of starch-sugar by means of methyl alcohol which produces no precipitate with genuine honeys, including both the ordinary form and the dextrorotatory variety. When starch-sugar is present there is a marked precipitate which should give the characteristic red colouration of erythrodextrin with iodine. The test has been extended so as to apply also to solid starch-sugar as follows: 5 c.c. of a 20% solution of of honey in water are mixed with 3 c.c. of 2% barium hydroxide solution and 17 c.c. of methyl alcohol and the mixture is well shaken. Pure honey remains clear, but the above adulterants cause a considerable precipitate. Methyl alcohol of high purity must be used.

This method has been applied quantitatively, but is of doubtful accuracy in such cases. However, it does enable the analyst even under unfavourable conditions to recognise the addition of small quantities of dextrin, starch-sugar or its syrup to conifer honey containing as much as 4 per cent. of natural dextrinous matter. (This subject is dealt with in more detail in Leffmann and Beam's Food Analysis.)

According to Leffmann and Beam, a common method of adulteration consists in pouring starch-sugar syrup over honey-comb from which the honey has been already drained. On standing such a mix-

ture acquires a honey flavour; it will give a high dextrorotatory polarisation hardly altered on hydrolysis.

The dextrin-like body in coniferous honey has been shown by König and Hörmann (*Zeit. Nahr.-Genussm.*, 1907, **13**, 113 to 132) to differ from dextrins prepared from starch by malt extracts or acids. It is fermented by beer yeasts.

The reliable points in the differentiation of honey-dew honeys and those adulterated with glucose are (*a*) the difference in invert polarisation between 20° and 87° (corrected to 77% of invert sugar); (*b*) the reaction of the honey and its precipitated dextrin toward iodine; (*c*) the polarisation of the inverted solution after precipitation of the dextrin with absolute alcohol. This process due to König and Karsch (*Zeit. anal. Chem.* 1895, **34**, 1) depends on the fact that after precipitating the dextrins and inverting natural honeys will show a lævorotation, honeys with 25% or more of glucose a dextrorotation.

NOTE.—To do this the difference in invert polarisation between 20° and 87° is multiplied by 77, the average percentage of invert sugar in pure honey, and this product divided by the percentage of invert sugar after invertion found in the sample. To find the percentage of pure honey in the sample this quotient is multiplied by 100 and divided by 26.7.

MAPLE PRODUCTS.

Maple Syrup[1] and Maple Sugar are products of considerable importance in the United States, but have not yet come to England in any quantity. They contain sucrose with minute amounts of special flavours and are frequently adulterated with sucrose from other sources or starch-sugar. The latter is detected by polarimetric examination before and after hydrolysis when pure maple sugar is inverted and has a negative rotatory power, glucose is but slightly affected.

Leffmann and Beam quote the following results obtained by Ogden:

	Polarimeter reading		Per cent. sucrose
	Direct	After hydrolysis	
Maple syrups free from glucoses,	53.1	—22.2	56.0
	59.6	—21.9	60.6
Maple sugars,	84.1	—28.8	85.9
	88.0	—28.3	87.6
Maple syrups containing glucoses,	80.0	+18.9	
	100.0	+45.6	

[1] Maple Syrup is defined to contain not more than 32% of water and not less than 0.45% of ash.

Pure maple syrup gives an abundant flocculent precipitate with methyl alcohol which does not adhere to the glass. When much starch-sugar is present a more granular precipitate is obtained which adheres to the glass.

Leach (Bulletin 65) finds that the grade of starch-sugar syrup commonly used to adulterate maple syrup, honey, molasses, etc., has a value $[a]_D = 87.5$ with a half-normal weight in a 2 dm. tube. He calculates approximately the amount present (G) from the formula $G = 0.561 (a - S)$, in which S = sucrose and a the polarimeter reading before hydrolysis. The sucrose is determined in the usual manner by the change in rotation on hydrolysis.

To judge the addition of sucrose the amount and alkalinity of the ash are carefully determined, also the amount of basic lead acetate precipitate and the malic acid value. The ash should not be less than 0.625 of the total sucrose; it should be determined by burning in a muffle at a low temperature as some of the constituents are volatile; it is also deliquescent and must be weighed quickly. The alkalinity of the water-soluble portion to phenolphthalein and methyl-orange and of the insoluble portion can be determined in the usual manner.

The malic-acid value is obtained as follows: 6.7 grm. of the sample are diluted with water to 20 c.c., the solution made slightly alkaline with ammonium hydroxide, 1 c.c. of 10% calcium chloride solution and 60 c.c. of 95% alcohol added and the beaker covered and heated an hour on the water-bath. After standing overnight, the precipitate is filtered through a hardened filter-paper, washed with 75% alcohol to remove all calcium chloride, dried and ignited. 20 c.c. N/10 hydrochloric acid are added, the lime dissolved by warming the solution and the excess of acid determined by titration. 0.1 of the number of c.c. of acid neutralised gives the provisional malic-acid value which should not be below 0.80 with pure maple products.

Horvet (J. Amer. Chem. Soc., 1904, 26, 1523) measures the volume of the lead precipitate after concentration by a centrifuge. Adulterated articles give much less precipitate.

Winton and Kreider (J. Amer. Chem. Soc., 1906, 28, 1204), instead of measuring the volume of the precipitate produced by adding basic lead acetate to maple products, determine the amount of lead in this precipitate. The lead numbers vary from 1.2 to 1.77 for genuine syrups and 1.8 to 2.5 for sugars.

The following tests, due to Sy (*J. Amer. Chem. Soc.*, 1908, 30, 1429-1431, 1611-1616), are stated to be of considerable value.

Colour Test.—15 c.c. of syrup or 15 grm. of sugar and enough water to make 15 c.c. are very thoroughly mixed in a test-tube with 3 c.c. of pure amyl alcohol and 1 c.c. of a 20% solution of phosphoric acid and allowed to stand until the alcohol separates. The alcoholic layer shows a decided brown colour with pure maple products; with adulterated samples the colour varies from a very pale to a light brown, according to the proportion of maple product; cane products containing caramel give no colour.

Foam Test.—5 c.c. of syrup are treated in a narrow tube graduated to 0.1 c.c. with 10 c.c. of water, the mixture is thoroughly shaken for half a minute and allowed to stand for 10 minutes, when the volume of foam is read. With pure maple products the amount is never less than 3 c.c.; adulterated products all give less.

Volume of Basic Lead Acetate Precipitate.—5 c.c. of syrup or 5 grm. of sugar and water to make 5 c.c. are placed in a glass-stoppered 25 c.c. measuring cylinder with 10 c.c. of water and 2 c.c. of 10% basic lead acetate solution. The whole is thoroughly mixed and allowed to settle for 20 hours when the volume of the precipitate is read. For pure maple products this will be over 3 c.c. and is usually over 5 c.c.; with adulterated products the volume is less.

A valuable criterion of the purity of a maple product is the *lead value* defined as the amount of lead precipitated on adding lead acetate solution to 100 c.c. of syrup or 100 grm. of sugar. 50 c.c. of syrup or 50 grm. of sugar are heated to boiling with 200 c.c. water to expel any fermentation products, 20 c.c. of 10% *normal* lead acetate added and again boiled. The solution is filtered when cold and the precipitate washed with water at 20°. The filter and precipitate are treated with 15 c.c. of concentrated nitric acid and 10 c.c. of concentrated hydrochloric acid, heated to disintegrate the filter, cooled, 10 c.c. concentrated sulphuric acid added, and the whole heated to expel all nitric acid. If the blackening does not disappear, 5 c.c. more nitric acid are carefully added after cooling and the whole is again heated to expel all nitric acid. The cooled solution is diluted with 50 c.c. water, cooled and 100 c.c. alcohol added. After 6 hours, the lead sulphate is filtered on an asbestos-packed gooch, washed with alcohol and ignited at a low red heat and weighed. The weight multiplied by 1.366 (2 x 0.683) gives the lead value which should

not be less than 0.25 and is usually over 0.30. It will be noted that this method differs from analagous methods now in use by the substitution of normal lead acetate for the basic salt.

GLUCOSIDES.

The name glucoside is applied to a numerous class of substances occurring in plants and seeds which yield a glucose, $C_6H_{12}O_6$, generally dextrose, on hydrolysis with acids. The other constituents yielded by glucosides are numerous, comprising alcohols, phenols, most of the vegetable dye-stuffs, mustard oil, many poisons, etc. A class of glucosides of some industrial importance since they are poisonous and yield hydrogen cyanide on hydrolysis are known as cyanogenetic glucosides. They have been found in many fodder plants. Most of the glucosides are accompanied in the plant by an enzyme capable of hydrolysing them. Enzyme and glucoside are stored in different cells in the plant, but act on one another when the plant is macerated with water. To extract a glucoside, therefore, it is first necessary to destroy the enzyme; this is often effected by boiling the plant with alcohol. Whilst the action of the enzyme is very specific and limited, as a rule, to a few closely related compounds, very many of the glucosides which are β-dextrose derivatives are hydrolysed by emulsin, an enzyme very widely distributed in plants and conveniently prepared from almonds.

The detection and determination of a glucoside in plant products is often best performed on the non-sugar constituent. Provided cane sugar or other disaccharides are not present, the change in cupric reducing power on hydrolysis by acids may be made use of. It is often preferable to extract the glucosides by means of solvents and weigh as such.

Bourquelot (*Arch. Pharm.*, 1907, **245**, 172) has elaborated a method for their detection by means of emulsin which he prepares by crushing · blanched almonds in a mortar and macerating each 100 grm. with 200 c.c. of distilled water containing chloroform for 24 hours at the normal temperature. The mixture is strained, pressed, protein precipitated by 10 drops of glacial acetic acid and, after filtering, the enzyme precipitated by 95% alcohol. The enzyme is collected, washed with a mixture of equal volumes of ether and alcohol and dried *in vacuo*. It is obtained as a white powder.

To detect glucosides, the material is first boiled with 95%

alcohol, any acidity neutralised with calcium carbonate, the spirit is distilled off and the residue made up to 250 c.c. with water containing a little thymol. Since the emulsin sometimes contains invertase, the material is first treated with invertase to eliminate sucrose, as described on page 314, heated to boiling for a few minutes to destroy the invertase and then treated with the emulsin preparation. The optical rotation is ascertained before and after hydrolysis; a change, if observed, denotes the presence of a β-glucoside of dextrose.

The problem often presents itself which of the glucose sugars is present in a glucoside. Ter Meulen (*Rec. Trav. Chim.*, 1905, **24**, 444) applies the principle, independently discovered by E. F. Armstrong (*Proc. Roy. Soc.*, 1904, **73**, 516), that the rate of action of a particular enzyme is hindered only by that sugar the glucosidic derivative of which is hydrolysed by the enzyme.

By measuring the rate of hydrolysis of the glucoside by its enzyme in the presence of various other sugars one only is found to retard, and this is the sugar present in the glucoside.

To ascertain whether a glucoside is a derivative of a- or β-dextrose, E. F. Armstrong hydrolyses with an active enzyme for half an hour and determines the change in optical rotatory power produced by the addition of a drop of aqueous ammonia. A decrease denotes the presence of a-dextrose, an increase that of β-dextrose. Dunstan, Henry and Auld (*Proc. Roy. Soc.*, 1907, B-**79**, 315, 322) have applied this method with success to the identification of a-dextrose in phaseolunatin.

Cyanogenetic glucosides are best determined by means of the hydrogen cyanide they produce when hydrolysed, as it is the poisonous nature of the feeding stuffs in which they occur which is really in question. Henry and Auld (*J. Soc. Chem. Ind.*, 1908, **27**, 428) have quite recently described the following method of procedure which gives the maximum amount of hydrocyanic acid obtainable. The product is ground as rapidly as possible, weighed, placed in a Soxhlet extraction apparatus and repercolated with hot alcohol so as to dissolve out the glucoside. The solvent is distilled off and the residue mixed with 50 c.c. of water and 10 c.c. of 10% hydrochloric or sulphuric acid added. The mixture is then distilled preferably in a current of steam until hydrocyanic acid can no longer be found in the distillate in which it may be estimated volumetrically by Liebig's method. The authors prefer to add a slight excess of sodium hydrogen carbonate and

titrate with an excess of iodine solution. A little of the freshly ground product is macerated with water in presence of an antiseptic to ascertain whether hydrogen cyanide is formed, thereby denoting the presence of the enzyme.

URINE ANALYSIS.

Sugars in urine may be detected and estimated either polarimetrically or by means of their reducing power or by the other usual methods elsewhere described. Normal urine at the most contains only traces of dextrose, but on the other hand, traces of substances other than glucoses, such as creatinine and uric acid, also glucuronic acid are normally present, all of which reduce Fehling's solution. Moreover, other optically active substances are generally present, so that urine requires a preliminary treatment before applying the sugar tests and it is necessary to make confirmatory tests to be sure sugar is present. Too great a stress must not be laid on the presence of an insignificant proportion of sugar. It is desirable before applying the sugar tests to remove proteins, if present, by adding a few drops of acetic acid, heating to boiling and filtering from any precipitate formed.

The liquid should then be rendered distinctly alkaline by sodium hydroxide, filtered from any precipitate, and the copper solution employed in the following manner:

Heat to boiling in a test-tube 10 c.c. of Fehling's solution, prepared in the usual way, previously introducing a few small fragments of clay tobacco-pipe to prevent bumping. When boiling, add 0.5 to 1 c.c of the urine, previously treated as indicated above. If sugar is abundant, a yellowish or brick-red opacity and deposit will be produced. If a negative reaction is obtained, test for traces of sugar by adding 7 c.c. or 8 c.c. of the urine to the hot liquid, heating again to ebullition, and then setting the tube aside for some time. If no turbidity is produced as the mixture cools, the urine is either quite free from sugar or at any rate contains less than 0.025%. If the quantity of sugar present is small—that is, under 0.5%—the precipitation of the yellow or red cuprous oxide does not take place immediately, but occurs as the liquid cools, the appearance being somewhat peculiar. The liquid first loses its transparency, and passes from a clear bluish-green to an opaque, light-greenish colour. This green milky appearance is quite characteristic of dextrose.

The colours of the precipitates obtained are attributed by some authors to the proportion of alkali present, the yellow and green precipitates being forms of cuprous hydrate. In other cases the disturbance is said to be due to the presence of creatinine. On adding Fehling's solution to a solution of this substance, a green liquid is produced, and on boiling a yellow colouration is observed, without, however, any separation of cuprous oxide. It is this behaviour which causes interference with the detection of glucoses, the combination of the yellow and blue colours resulting in a green, and in addition the creatinine compound is said to have the power of preventing the precipitation of cuprous oxide by glucoses.

Nylander's test which is not affected by creatinine or uric acid consists in boiling the urine for 2 to 5 minutes with a small quantity of a solution containing 100 grm. of sodium hydroxide (sp. gr. 1.12), 4 grm. of potassium sodium tartrate and 2 grm. of bismuth subnitrate, when a black precipitate forms on cooling.

The detection and estimation of sugars in urine offers, but little difficulty when the amount is 0.25% or over, but when the quantity is very small satisfactory results are not often attainable. The occurence of sugar normally in the urine has been much disputed. By the use of phenylhydrazine—a method free from the objections and fallacies which underlie nearly all other tests—it seems proved that, while normal human urine may sometimes contain traces of sugar, that substance is by no means constantly present, and a great number of the recorded observations are quite inconclusive.

It is important to consider the extent to which these bodies interfere, and the manner in which they may be removed or their influence obviated. The chief of these are uric acid, xanthine, and creatinine, but under some conditions urine contains glucuronic acid or compounds thereof which simulate sugar very closely. The amount of uric acid passed per diem under ordinary conditions is said to be about 0.5 grm., though, of course, in many instances it is considerably more. Xanthine and the allied bodies are present in still smaller amount. According to Voit, the proportion of creatinine passed in 24 hours ranges from 0.5 to nearly 5 grm. Urine containing the latter amount would exert a reducing action on Fehling's or Pavy's solution equivalent to the presence of 0.32% of glucose.

Dextrose in urine may be estimated by:

1. Titration with Fehling's solution in the usual manner.

2. Titration with Pavy's solution.
3. Titration with Knapp's mercurial solution.
4. Polarisation.
5. Fermentation.

For ordinary clinical purposes very great accuracy is not required. In such cases as the proof of a diminution in the amount of sugar following treatment, the errors of collecting urine properly and multiplication so as to give the daily output, more than counterbalance slight errors in the estimation.

Before polarising, the urine may be clarified and freed from proteins, uric acid, phosphates and colouring matters by precipitating with alumina cream or with basic lead acetate. Thus, 100 c.c. of urine of known sp. gr. are measured into a flask, 5 c.c. of the clarifying reagent added, the solution made up to 110 c.c., shaken, filtered and polarised. To make certain that the rotation obtained is really due to dextrose, the urine is examined polarimetrically before and after fermentation, the change in rotatory power indicating the amount of dextrose present.

The fermentation test for dextrose in urine is very useful for confirmatory purposes and also serves to distinguish between dextrose and unfermentable pentoses, lactose or glucuronic acid. Fermentation at 34 to 36° should be complete in 6 hours. If the operation be prolonged the gas formed is probably due to other changes. The Lohnstein saccharometer is often used in physiological laboratories for this purpose.

Fehling's solution is used either gravimetrically or volumetrically in the ordinary manner.

Modified Fehling Solution.—For examination of urine the following modification of the copper solution is strongly recommended by S. R. Benedict (*J. Biol. Chem.*, 1909, 5, 485):

Copper sulphate (cryst.), 8.65 grm.
Sodium citrate,   86.50 grm.
Sodium carbonate (dry), 50.00 grm.

The sodium citrate and carbonate are dissolved in 300 c.c. of water, filtered if necessary, and made up to 425 c.c. The copper sulphate is dissolved in 50 c.c. of water and made up to 75 c.c. The solutions are mixed. The mixture keeps well. Benedict found that commer-

cial sodium citrate is satisfactory. The solution is not reduced by uric acid, chloroform, chloral, or formaldehyde.

Pavy's solution may also be used for the determination of the glucose in diabetic urine, though it cannot be employed for the detection of small quantities of the sugar. Müller and Hagen determine the sugar volumetrically by Knapp's mercurial solution, which has the advantage of being applicable to samples of urine containing as little as 0.1% of glucoses, while Fehling's solution cannot be applied quantitatively in the ordinary manner if less than 0.5% of dextrose be present, owing to the incomplete separation of the cuprous oxide in presence of certain obscure foreign matters contained in urine.[1]

To render urine fit for the application of Fehling's solution, Carnelutti and Valente recommend that 100 c.c. of the sample should be evaporated to a syrup on the water-bath, 1 c.c. of a 25% solution of zinc chloride previously mixed with 1/4 of its volume of hydrochloric acid is added, then 2 volumes of absolute alcohol, and the whole allowed to stand for some hours. The liquid is then filtered, the residue washed with alcohol, the alcohol evaporated from the solution, and the residual liquid made up to 100 c.c. with distilled water. In this solution excellent results are said to be obtainable by Fehling's solution.

Copper sulphate yields at first little or no precipitate with normal urine in the cold, but on standing or boiling a pale green precipitate is thrown down which has a tendency to darken if the heating be continued. If copper acetate be used, or sodium acetate with copper sulphate, the precipitation is more complete, uric acid, xanthine, hypoxanthine, colouring matter, and albumin being entirely thrown down, and creatinine and phosphates partially. The filtered liquid cannot be used for the phenylhydrazine test, and the presence of copper unfits it for titration by Pavy's solution; but it is admirably suited for the detection of small quantities of sugar by Fehling's test, as follows:

From 7 to 8 c.c. of the sample are heated to boiling, and, without separating any precipitate of proteins, 5 c.c. of the solution of copper sulphate used for preparing Fehling's test are added and the liquid again boiled. This produces a precipitate principally uric acid, xanthine, hypoxanthine, and phosphates. To render the precipitation com-

[1] J. G. Otto recommends that, for titrating solutions containing 1 to 0.5% sugar, the Knapp's solution should be diluted with 4 volumes of water, for those containing 0.5 to 0.1%. with 3 volumes of water, while for solutions containing less than 0.1% 2 volumes of water should be added. In all cases the urine should be added gradually to the mercurial solution.

plete, however, it is desirable to add to the liquid, when partially cooled, from 1 to 2 c.c. of a saturated solution of sodium acetate having a feebly acid reaction. The liquid is filtered, and to the filtrate, which will have a bluish-green colour, 5 c.c. of the alkaline tartrate mixture used for preparing Fehling's solution are next added, and the liquid boiled for fifteen to twenty seconds. In the presence of more than 0.25% of sugar, separation of cuprous oxide occurs before the boiling point is reached, but with smaller proportions precipitation takes place during the cooling of the solution, which becomes greenish, opaque, and suddenly deposits cuprous oxide as a fine orange-yellow precipitate. When a urine rich in sugar is under examination, the volume taken can be advantageously reduced from 7 or 8 c.c. to 2 or 3 c.c. or even less, water being added to replace it.

It is evident that in this modification of the ordinary Fehling's test advantage is taken of the very general precipitating power of cupric acetate to remove from the urine the great majority of those substances which interfere with the detection of sugar, by themselves reducing the alkaline copper solution, retaining the cuprous oxide in solution, or producing a flocculent precipitate which masks the true reaction of sugar. Operating as described above, no greenish turbidity refusing to settle is produced, and hence the separation of any cuprous oxide is very readily observed. It is important that the sodium acetate should not be added till the liquid has partially cooled, so as to avoid any chance of reaction of the resultant cupric acetate with the glucose in the manner observed by Barfoed.

Pavy's method of estimating sugar by titration with ammoniacal cupric solution would probably be more generally applied if it did not necessitate the use of a special apparatus. The following form of the test is simple and covenient, but less accurate than where larger quantities of the urine and reagent are employed. An accurately measured volume of 10 c.c. of Pavy's solution is placed in a wide test-tube, a few fragments of tobacco-pipe dropped in, and 8 to 10 drops of petroleum or paraffin burning oil added. This forms an upper layer which effectually excludes the air. The test-tube is inserted into the neck of a wide-mouthed flask containing hot water, which is then heated until the contents of the tube have reached the point of ebullition. The urine to be tested is treated with an equal volume of ammonia and filtered from the precipitated phosphates. A known volume of the filtrate is then further diluted with a definite quantity of water,

according to the proportion of sugar supposed to be present, and then added drop by drop to the boiling-hot Pavy's solution by means of a small burette or graduated pipette, until the disappearance of the blue colour indicates the termination of the reaction. If 10 c.c. of Pavy's solution were employed, the volume of urine required to decolourise it contains 0.005 grm. of sugar.

Unclarified healthy human urine may exert a reducing action on Pavy's solution equal to that of a liquid containing from 0.1 to 0.3% of dextrose. Of this, one-quarter is ascribed to uric acid (removable by lead acetate) and the remainder to creatinine (removable by mercuric chloride).

The phenylhydrazine test for dextrose has a special value as it is not given by the other non-sugar reducing substances in the urine. To apply it, 50 c.c. of the suspected urine previously freed from protein, are heated in the boiling water-bath for an hour, with 10 to 20 drops of phenylhydrazine and the same volume of 50% acetic acid. 5 grm. of sodium chloride may be added to facilitate precipitation. If any quantity of dextrose is present, an orange-yellow, generally crystalline, precipitate separates in the hot liquid or on cooling. This should be filtered when cold, well washed with water to remove excess of phenylhydrazine and crystallised from a small quantity of dilute alcohol when characteristic yellow needles are obtained, melting at 205° and practically insoluble in boiling water.

When only minute traces of sugar are present, the complete separation of the glucosazone requires some time, but the qualitative indication is readily and quickly obtained. Dextrose and lævulose yield the same glucosazone, the pentoses and glucuronic acid (see later) also yield insoluble compounds with phenylhydrazine.

Is is important that the phenylhydrazine used should be of good quality. It should be almost straw-yellow in colour and is conveniently kept in sealed bottles containing only a small quantity which can be quickly used when once opened.

Salkowski takes 5 c.c. of urine, 0.5 c.c. of glacial acetic acid and 20 drops of phenylhydrazine, boils for 1 minute, adds 5 drops of 15% sodium hydroxide, and a volume of water equal to 3/4 of the original volume and heats nearly to boiling. After standing 24 hours, a sulphur-yellow precipitate of slender needles is obtained if dextrose were present, but not from lactose or maltose.

Unfortunately, the phenylhydrazine test cannot be applied quanti-

tatively though the amount of precipitate formed gives a fair indication of the proportion of sugar present.

Glucuronic Acid.—As already stated glucuronic acid simulates the behaviour of dextrose very closely and gives not only all the ordinary reactions as a reducing agent, but is the only other constituent of urine which reacts with phenylhydrazine.

Glucuronic acid is a syrupy liquid, miscible with alcohol or water. When the aqueous solution is boiled, evaporated, or even allowed to stand at the ordinary temperature, the acid loses the elements of water and yield the anhydride or lactone ($C_6H_8O_6$), which forms monoclinic tables or needles, having a sweet taste and melting at 167°. The lactone is insoluble in alcohol, but dissolved by water to form a solution which is dextrorotatory ($[a]_D = 19.25°$), prevents the precipitation of cupric solutions by alkalies, and powerfully reduces hot Fehling's solution, the cupric reducing power being 98.8 compared with dextrose as 100. The acid is dextrorotatory ($[a]_D = 35°$), but many of its compounds are lævorotatory. It reduces Fehling's solution on heating, and precipitates the metals from hot alkaline solutions of silver, mercury, and bismuth. With phenylhydrazine, glucuronic acid forms a yellow crystalline compound, melting at 114 to 115°, and resembling closely phenylglucosazone. When oxidised with bromine glucuronic acid yields saccharic acid, which can be again reduced to glucuronic acid by treatment with sodium amalgam. It is distinguished from glucose by not undergoing the alcoholic fermentation when treated with yeast.

It gives the orcinol and phloroglucinol reactions for pentoses as it is dehydrated on boiling with hydrochloric acid, yielding furfural and carbon dioxide, but the production of furfural is much slower than in the case of the pentoses. The carbon dioxide evolved may be used to estimate glucuronic acid. To effect this, Lefevre and Tollens (*Ber.*, 1907, 40, 4513) boil with hydrochloric acid (sp. gr 1.06) for 3 1/2 hours and aspirate a current of pure air through the apparatus; the carbon dioxide is washed and absorbed in potash bulbs and weighed. The results are, as a rule, too high owing to the presence of other substances which yield carbon dioxide.

Combined with the determination of the furfural, this method affords a simultaneous estimation of pentoses and glucuronic anhydride. Three parts of glucuronic anhydride give one part of furfural phloroglucide.

Tollens (Ber., 1908, **41**, 1788–1790) finds that glucuronic acid alone and not the pentoses form a blue substance on heating with naphthoresorcinol and hydrochloric acid which is soluble in ether. This enables glucuronic acid to be identified with certainty in presence of pentoses.

Other Sugars in Urine.—Recent researches have shown the occasional presence of other sugars besides dextrose in pathological urine. The reducing action of a urine may indicate dextrose, lævulose, lactose, pentoses or glucuronic acid, the fermentation test only dextrose and lævulose.

These sugars are best detected by means of the substituted phenyl-hydrazines.

To detect pentoses in urine, the orcinol test is carried out as follows. 0.03 grm. of powdered orcinol are dissolved in 10 c.c. of fuming hydrochloric acid and a drop of dilute ferric chloride added. 5 c.c. of this solution and 2 c.c. of urine are placed in a tube closed with a plug of cotton wool and heated nearly to boiling. If pentoses are present an emerald-green colouration gradually appears, which soon becomes dark green.

To estimate pentoses in urine, Jolles (*Zeit. anal. Chem.*, 1907, **46**, 764) proceeds as follows: The urine is boiled with a few drops of acetic acid and concentrated, if necessary, to free it from interfering volatile substances. 100 c.c. are distilled with 150 c.c. hydrochloric acid (sp. gr. 1.06) in a current of steam until the distillate amounts to 1000 c.c. 100 c.c. of this are neutralised with an excess of 20% sodium hydroxide, using methyl-orange as indicator; half-normal hydrochloric acid is added to restore the red colouration and the liquid titrated with sodium hydrogen sulphite and standard iodine solution.

For further information consult "*Analyse des Harns.*"

PENTOSES.

The best known pentoses are arabinose and xylose. Even more important are their polymerides—the pentosans.

Arabinose and xylose, in the absence of other sugars, may be detected and estimated in the same manner as dextrose—either polarimetrically or by means of copper or mercuric solutions.

Characteristic are the colourations produced with alcoholic solutions of phenols and hydrochloric or sulphuric acid on cautious heating.

Orcinol gives a bluish-violet colouration in the cold, and on warming, a reddish, changing to violet-blue and finally bluish-green flakes separate which dissolve in alcohol, showing a characteristic absorption-spectrum.. The orcinol reagent is prepared by dissolving 1 grm. of orcinol in 200 c.c. of 94% alcohol. 3 drops are added to 5 c.c. of the sugar solution and then 5 c.c. of concentrated hydrochloric acid. The mixture is shaken and heated on a boiling water bath for half an hour. The method is not available in presence of laeonlose when a bronze brown colouration is obtained (Pieraerts. *Bull. Assoc. Chim. Sucr. et Dist.*, 1908, **26**, 46).

Phloroglucinol and hydrochloric acid cause a bright cherry-red on heating and the solution so prepared has a very characteristic spectrum.

A more general method, which is applicable also to the pentosans, consists in distillation with hydrochloric acid in a current of steam whereby furfural is formed and estimated by means of its compounds with phloroglucinol, phenylhydrazine or sodium hydrogen sulphite.

Of these the phenylhydrazine method is least satisfactory. When using sodium hydrogen sulphite, an aliquot portion of the distillate is mixed with a known volume in excess of the standard hydrogen sulphite. After two hours' standing, this excess is estimated by titration with standard iodine solution: 1 c.c. of normal sodium hydrogen sulphite solution is equivalent to 0.07505 grm. of pentose.

The phloroglucinol method has been worked out in great detail by Kröber (*J. Land.*, 1900, **48**, 379) and adopted by the A. O. A. C. The details are as follows:

Qualitative Test of the Purity of the Phloroglucinol. —Dissolve a small quantity of the phloroglucinol in a few drops of acetic anhydrid, heat almost to boiling, and add a few drops of concentrated sulphuric acid. A violet colour indicates the presence of diresorcinol. A phloroglucinol which gives more than a faint colouration may be purified by the following method: Heat in a beaker about. 300 c.c. of hydrochloric acid (sp. gr. 1.06) and 11 grm. of commercial phloroglucinol, added in small quantities at a time, stirring constantly until it has almost entirely dissolved. Some impurities may resist solution, but it is unnecessary to dissolve them. Pour the hot solution into a sufficient quantity of the same hydrochloric acid (cold) to make the volume 1500 c.c. Allow it to stand at least overnight—better several days—to allow the diresorcinol to crystallise out, and filter immediately before using.

The solution may turn yellow, but this does not interfere with its usefulness. In using it, add the volume containing the required amount to the distillate.

Procedure.—Place a quantity of the material, chosen so that the weight of phloroglucide obtained shall not exceed 0.300 grm. in a flask, together with 100 c.c. of 12% hydrochloric acid (sp. gr. 1.06), and several pieces of recently heated pumice stone. Place the flask on a wire gauze, connect with a condenser, and heat, rather gently at first, and so regulate as to distill over 30 c.c. in about ten minutes, the distillate passing through a small filter-paper. Replace the 30 c.c driven over by a like quantity of the dilute acid added by means of a separating funnel in such a manner as to wash down the particles ad-hering to the sides of the flask, and continue the process until the distillate amounts to 360 c.c. To the completed distillate gradually add a quantity of phloroglucinol (purified if necessary) dissolved in 12% hydrochloric acid and thoroughly stir the resulting mixture. The amount of phloroglucinol used should be about double that of the furfural expected. The solution first turns yellow, then green, and very soon an amorphous greenish precipitate appears, which grows rapidly darker, till it finally becomes almost black. Make the solution up to 400 c.c. with 12% hydrochloric acid, and allow to stand overnight.

Filter the amorphous black precipitate into a tared gooch crucible through an asbestos felt, wash carefully with 150 c.c. of water in such a way that the water is not entirely removed from the crucible until the very last, then dry for four hours at the temperature of boiling water, cool and weigh, in a weighing bottle, the increase in weight being reckoned as phloroglucide. To calculate the furfuraldehyde, pentose, or pentosan from the phloroglucide use the following formulæ given by Kröber:

a. For weight of phloroglucide "a" under 0.03 grm.

Furfural $= (a + 0.0052) \times 0.5170$.

Pentoses $= (a + 0.0052) \times 1.0170$.

Pentosans $= (a + 0.0052) \times 0.8949$.

b. For weight of phloroglucide "a" over 0.300 grm.

Furfural $= (a + 0.0052) \times 0.5180$.

Pentoses $= (a + 0.0052) \times 1.0026$.

Pentosans $= (a + 0.0052) \times 0.8824$.

For weight of phloroglucide "a" from 0.03 to 0.300 grm. use Kröber's table (*J. Landw.*, 1900, **48,** 379), or the following formulæ:

$$\text{Furfural} = (a + 0.0052) \times 0.5185.$$
$$\text{Pentoses} = (a + 0.0052) \times 1.0075.$$
$$\text{Pentosans} = (a + 0.0052) \times 0.8866.$$

Methyl pentoses (such as rhamnose) are estimated volumetrically in the same way as pentoses, the conversion into methylfurfural taking place quantitatively on distillation of a methylpentose with hydrochloric acid in a current of steam.

In a mixture of pentoses and methylpentoses, the total sugar is determined by distillation with hydrochloric acid; in a second portion of the sample the methylpentoses are precipitated by alcohol and saturated aqueous baryta at 0° and the pentoses are estimated in the filtrate.

STARCH AND ITS ISOMERIDES.

E. FRANKLAND ARMSTRONG, D. Sc , Ph. D., A. C. G. I.

In the vegetable kingdom, and to a minor extent in the animal kingdom, there exist a number of carbohydrates having in common a composition represented by the empirical formula $C_6H_{10}O_5$, but their physical and chemical characters point in all cases to a multiple of this formula as the true representation of the constitution of the molecule.

The carbohydrates of the starch group are non-volatile bodies, and, with perhaps one or two exceptions, are amorphous. As a class they are insoluble in alcohol, though the greater number of them are dissolved by water, forming solutions which usually exert a marked rotatory action on a ray of polarised light. They are neutral in reaction, and form but few definite compounds or metallic derivatives. They are very numerous, and apparently capable of isomeric modification. Owing to their physical characters and feebly-marked chemical affinities, it is often difficult to obtain them in a state of purity.

None of the members of the group reduces Fehling's solution when boiled with it. By treatment with acids they undergo hydrolysis, yielding sugars among other products, and then reduce the cupric solution.

Many of the members of the group are of little practical interest, and their analytical reactions have been very incompletely studied. The following table serves to show the comparative characters of the more important members, and cellulose, starch, and dextrin are described more fully in subsequent sections. Elsewhere will be found tables for the general proximate analysis of plant-products, and under the head of "Gums" a short description of pectinous matters.

Name.	Empirical formula.	Chief sources and modes of formation.	Specific rotatory power.	Solubility in water.	Products obtained by boiling with dilute acid.	Reaction with iodine solution.	Other characters.
Cellulose.	$C_6H_{10}O_5$	Cotton-wool; filter-paper; linen-rags.	.....	Not soluble.	Not changed	No change; blue in presence of zinc chloride.	Soluble in Schweitzer's reagent, forming levo-rotatory solution. With strong sulphuric acid, followed by dilution, gives dextrose, etc.
Starch,	$C_6H_{10}O_5$	Amylaceous seeds, roots, etc.	$[\alpha]_D = +200$	Insoluble, cold; gelatinised and dissolved on boiling.	Maltose and dextrin; ultimately dextrose.	Violet-blue.	appearance n Schweitzer's tannin and
Glycogen,	$C_6H_{10}O_5$	Liver of man and herbivorous animals; oysters.	$[\alpha]_D = +197°$	Slowly soluble; solution opalescent, cleared by acetic acid.	Dextrose.	Wine-red.	oluble in al-
Inulin,	$C_6H_{10}O_5$	Elecampane, dahlia; dandelion, chiccory.	Levo-rotatory.	Cold, slightly soluble; hot, readily soluble.	Levulose.	No change.	White, hygroscopic powder, or sphæro-crystals. Insoluble in absolute alcohol, sparingly in dilute. Reduces ammonio-nitrate of silver.
Dextrin,	$C_6H_{10}O_5$	Action of acids or diastase on starch.	$[\alpha]_D = +200$	Readily soluble.	Dextrose.	Erythro-dextrin, reddish-brown; achrodextrin, colourless.	White, very deliquescent. Apparently two varieties, differing in their reaction with iodine. Insoluble in alcohol.
α-Amylan,	$C_6H_{10}O_5$	Barley, etc.	$[\alpha]_D = -34$	Cold, nearly insoluble; hot, gelatinises and dissolves sparingly.	Dextrose.	No change.	Amorphous, white substance.
β-Amylan	$C_6H_{10}O_5$	Wheat, rye, etc	$[\alpha]_D = -144$ to -148	Soluble in cold water, forming very viscous solution.	A dextro-glucose.	No change.	Amorphous, white substance. Fresh solution exhibits bi-rotation.
Gums,	.....	.....	Levo-rotatory.	Soluble in, or swollen by, cold water.	Hexoses and Pentoses.	No change.	Amorphous. Solutions highly colloid. Insoluble in alcohol. Yield mucic acid on treatment with nitric acid.

STARCH.

Starch is the characteristic product of the vegetable kingdom; it is formed in almost every part of plants. It is a white, glistening, tasteless powder, fixed in the air and not volatile nor crystallisable. It is very hygroscopic and contains when air-dried from 16 to 28% of water and still about 10% of water when dried in a vacuum.'

Starch is not dissolved without change by any known solvent and is quite unacted on by alcohol, ether or cold water. When heated with water to a temperature which differs slightly according to the origin of the starch,[1] the granules swell up and form a paste, and in presence of much water colloidal solutions are formed, the exact nature of which is still imperfectly understood. It is more than probable that it is not a single substance of very high molecular complexity, as generally supposed, but a mixture of closely related isomerides of somewhat simpler structure.

On hydrolysis with dilute acids, starch is converted into a mixture of dextrins and maltose; prolonged treatment results in further hydrolysis and ultimate complete conversion into dextrose. A solution of starch is hydrolysed by malt extract (*diastase*) to dextrin and maltose even in the cold; solid starch is not attacked by malt extract unless a liquefying enzyme be also present. The saliva ferment also hydrolyses starch.

Soluble starch is produced by boiling starch with water; the solution obtained may be rendered quite clear by the addition of a little caustic alkali. It is the first product of the action of dilute acids or ferments on starch. To prepare it the Malt Analysis Committee (see under Malt) advise digesting purified potato starch with dilute hydrochloric acid (sp. gr. 1.037) at 60–65° F. for 7 days.

Soluble starch is a very perfect colloid and has a high viscosity. It is strongly dextrorotatory.

Parow, Ellrodt, and Neumann (*Zeit. Spiritusind.*, 1907, **30**, 432) give the following mean results for the specific gravities of various starches.

[1] The gelatinisation temperatures are as follows:

Green malt and oat starch,	85° C.
Barley, kilned malt, rye, wheat and rice starch,	80° C.
Maize starch,	75° C.
Potato starch,	65° C.

Starch from:	Sp. gr. of anhydrous starch in:		Sp. gr. of hydrated starch in:			
			Water		Toluene	
	Water	Toluene	Per cent. of water in the starch	Sp. gr.	Per cent. of water in the starch	Sp. gr.
Potatoes,	1.648	1.513	18.72	1.463	15.03	1.361
			19.35	1.436		
			20.14	1.453		
Wheat,	1.629	1.502	13.38	1.515	13.90	1.365
			13.80	1.496		
			14.60	1.492		
Maize,	1.623	1.499	11.06	1.522	12.60	1.378
			12.88	1.504		
			14.36	1.490		
Rice,	1.620	1.504	11.92	1.514	14.03	1.360
			13.10	1.500		
			14.14	1.501		

Structure of Starch Corpuscles.—Starch occurs in plants in the form of minute granules, which generally possess a concentrically stratified structure similar to that of an onion. These granules consist chiefly of a body called granulose, together with a closely allied substance known as amylo-cellulose or starch-cellulose, and water and traces of mineral matter. Starch-cellulose occurs in largest proportion in the outer layers of the granule, and probably constitutes the whole of the external coating. Owing to this protective coating, starch granules are wholly unacted on by cold water, as the internal granulose, though slightly soluble, is highly colloidal. When the outer layer of the granule is ruptured, as by grinding the starch with sand, water acts readily on it, and the liquid gives an intense blue colour with iodine. By treating starch paste with malt-extract, the insoluble starch-cellulose may be obtained pure, and then is found to give only a dirty yellow colour with iodine. Saliva (owing to the ptyalin contained in it) and, at a temperature of 50° to 60° pepsin, organic acids, very dilute hydrochloric or sulphuric acid, and a saturated solution of sodium chloride containing 1% of hydrochloric acid, all dissolve out the granulose and leave the amylo-cellulose intact. By boiling with water, starch-cellulose is mostly converted into soluble starch,

leaving, however, a portion which obstinately resists the action of water, but is readily dissolved by dilute alkali. Amylo-cellulose differs from ordinary cellulose in being insoluble in Schweitzer's reagent. By repeated alternate treatment of potato-starch in the cold with very dilute alkali and acid, the cellulose may be removed, when the residue dissolves in hot water to form a perfectly clear solution. Solid starch corpuscles, when treated with iodine solution, are coloured intensely blue, the reagent readily penetrating the coating of cellulose and thus reaching granulose.

Young small corpuscles of starch appear to be invariably spherical, but as they grow older they may become lenticular, ovoid, or polygonal. The shape and size of the starch corpuscles are often highly characteristic of the plant by which they were produced, and this fact is frequently taken advantage of for identifying the presence of starch from particular sources.

Microscopic Identification of Starches.—When a sample is to be examined under the microscope for the identification of its starch, a minute quantity should be placed on a glass slide with the point of a knife. If in a powdered state, or readily reducible to powder, a preferable plan is to stir the sample with a dry glass rod, and tap the rod on the glass slide. A drop of distilled water or diluted glycerol (1 of glycerol to 2 of water) should then be added, and if the unpowdered structure be employed it should be broken up by careful crushing with the point of a knife. A glass cover is then put on, and any superfluous moisture removed by blotting-paper. The specimen is now ready for observation. Somewhat oblique light should always be employed, and the power should be about 200 diameters.

The points to be observed in the microscopic observation of starches are: (a) The shape and size of the granules. (b) The position and character of the *hilum*. (c) The concentric markings. (d) The appearance under polarised light. The first two observations are tolerably simple, but the examination for rings requires care, the markings being rarely visible without very cautious manipulation of the illumination and movement of the fine-adjustment, and then only in a few granules at the same time. Natal arrowroot and turmeric starches show well-developed rings on nearly every granule. Wheat, on the other hand, shows few rings, even in the best light. When the hilum is situated near the centre of the granule, the rings are usually complete.

but when the hilum is near one end of the granule only a segment of each ring is visible.

Although the size of starch-granules is a highly important character, it must be remembered that great difference is often noted between individual granules, and that it is only the general or average size of the corpuscles which is usually recorded. Difference in size of the starch-granules is very marked in the case of the potato, in which the corpuscles range from 0.0025 of an inch in length down to less than 0.0002 (0.063 millimetre to less than 0.005).

Examination with polarised light, either with or without the use of a selenite plate, is a valuable auxiliary means of identifying starches, but many of the statements made in books, such as the black cross being observable in the case of certain starches only, must be considered as merely applicable to the precise conditions under which the observations referred to were made. With proper manipulation, all starches appear to show the black cross, and an ignorance of this fact has led many into error. Some starches show much more colour than others when examined under the polarising microscope. For observation of starches by polarised light it is often desirable to employ a highly-refracting mounting medium, and for such purposes water may be advantageously replaced by diluted glycerin, glycerol jelly, Canada balsam, oil of anise, carbon disulphide, etc.

Much has been written on the microscopic appearance of starches, and some observers profess to be able to distinguish starch of almost every origin. To the observer who has not made a special study of the morphology of starches, these distinctions are in many cases wholly unrecognisable, and as the minute points of difference are almost incapable either of description or delineation, the only safe method of discriminating starches is by a careful comparison of the sample with specimens of known origin and purity, making the observations under exactly similar conditions as to illumination, magnifying power and mounting medium. These standard specimens should not be permanently mounted, but kept in an air-dry state, and a minute quantity mixed with water or other medium when required for use. As a rule, it is quite unnecessary to prepare the pure starches for comparision, a direct employment of the air-dried tissue answering every purpose.

Very complete tabular schemes for the recognition of starches by the microscope have been devised by Muter (*Organic Materia Medica*), but a later work is that of Greenish, *Microscopical Examination of Foods*

and Drugs. Of course, they in no way enable the observer to dispense with the requisite experience in observation, but they much facilitate the recognition by drawing attention to the more characteristic features of the starches. The figures of starch granules on pages 414 to 416 are derived from Greenish's book. They first appeared in the Pharmaceutical Journal.

The following arrangement of starches, according to their microscopic appearance, is based on that of Muter. The starches are arranged in 5 classes.[1]

I. **The potato group** includes such oval or ovate starches as give a play of colours when examined by polarised light and a selenite plate, and having the hilum and concentric rings clearly visible.

II. **The leguminous starches** comprise such round or oval starches as give little or no colour with polarised light, have concentric rings all but invisible, though becoming apparent, in many cases, on, treating the starch with chromic acid, while the hilum is well marked, and cracked or stellate.

III. **The wheat group** comprises those round or oval starches having both hilum and concentric rings invisible in the majority of granules. It includes the starches from wheat and some other cereals, and a variety of starches from medicinal plants, such as jalap, rhubarb, senega, etc.

IV. **The sago group** comprises those starches of which all the granules are truncated at one end. It includes some starches used for food, together with those from belladonna, colchicum, scammony, podophyllum, canella, aconite, cassia, and cinnamon.

V. **The Rice Group** contains the starches all the granules of which are polygonal in form. It includes the starches from oats, maize, buckwheat, rice, pepper, and ipecacuanha.

The following table gives further particulars respecting the microscopic appearance of the more important starches. The figures expressing the sizes are micro-millimetres (1/1000th millimetre,) but they may be converted into ten-thousandths of an inch by multiplying them by the factor 0.3937.

In the case of elongated starches, the figures expressing the size have reference to the mean of the longer and shorter diameters.

[1] In order that mistakes may not be made in differentiating starches by the scheme, it is important to bear in mind that the appearances described apply to the following conditions of examination, namely, observation with oblique light; use of water as a medium, and, when polarised light is used, the use of a red-green selenite plate with diluted glycerol as a mounting medium.

Origin of starch.	Diameter in micromillimetres.	Characteristic shape of granules.	Other characters.
CLASS I.			
Canna, or tous-les-mois.	47–132	Irregular oval, or oyster-shaped. (Fig. 59.)	Hilum annular and eccent·ic. Rings incomplete, very fine, narrow, and regular. Alkali develops lines and hilum. Well-marked and regular cross with polarised light.
Potato.	Very variable; usually between 60 and 100	Small granules, circular; the larger ovate, or oyster-shaped. (Fig. 60.)	Hilum, a spot, generally near smaller end. Rings in larger granules numerous and complete. Very distinct cross towards smaller end, and brilliant colours with polarised light.
Maranta-arrowroot.	10 to 70 average 36	Somewhat ovoid or mussel-shaped, tendingtotriangular in larger granules. Sometimes irregular, with a nipple-like projectionatsameend as hilum. (Fig.61)	Hilum, near one end, either circular or linear, and often cracked. Rings numerous and always visible, but not strongly marked. Well-defined cross towards larger end with polarised light, and brilliant colours.
Natal-arrowroot.	33 to 38	Broadly ovate, or occasionally circular, with irregular projections.	Hilum, a crack, eccentric. Rings very distinct under water.
Curcuma-arrowroot.	30 to 61	Resemblesmaranta. Elongated, or oval with irregular projection. (Fig. 62.)	Hilum, an eccentric dot or circle. Indistinct segments of rings. Heat or alkali deforms granules very irregularly.
CLASS II.			
Bean.	Nearly uniform 30 to 35	Reniform or oval.	Hilum, stellate. Often becoming a longitudinal furrow. Smaller granules predominate.
Pea.	Very variable 20 to 40	Reniform or oval.	Hilum elongated. Not distinguishable from bean in mixtures.
Lentil.	20 to 40	Reniform or oval.	Hilum elongated and very clearly defined. Rings moderately distinct.
CLASS III.			
Wheat.	Very variable 2 to 52	Circular or nearly so, and flattened. (Fig. 63.)	Chiefly of two sizes, large and very small. Shows a cross in glycerin with polarised light, but very slightly in water. Faint rings and colours are visible on the most elliptical granules.
Barley.	Fairly uniform 13 to 39	Closely resembles wheat; some granules slightly angular, or elliptical. (Fig. 64.)	Not certainly distinguishable from wheat in mixtures of the two.

Origin of starch	Diameter in micro-milli-metres.	Characteristic shape of granules.	Other characters.
Rye.	2 to 38	Closely resembles wheat. (Fig. 65.)	A few granules show a three- or four-armed fissure extending nearly to the circumference.
Oat.	....	Large oval granules showing polygonal divisions. (Fig. 66.)	The compound granules break up by attrition into polygonal granules (see Class V.).
Acorn.	19	Circular or slightly oval.	Eccentric hilum developed by chromic acid.
CLASS IV. Arum.	14	Truncated with two facets.	Hilum eccentric.
Tacca-arrowroot.	9 to 19	Resembles tapioca.	Distinct hilum, linear and often starred. Very varied shape, often resembling maize, but has sharp angles.
Sago.	25 to 66	Ovate, or truncated oval. (Fig. 67.)	Hilum, a circular spot or crack at convex end; faint rings. Well-defined cross, and often colours with polarised light. *Prepared sago* shows large oval depression; with polarised light characters less definite than the raw.
Tapioca.	8 to 22	Kettle-drum, or circular. (Fig. 68.)	Hilum, a dot or short slit, nearly central. Well-defined cross and colours with polarised light. Characters of *prepared tapioca* are less definite.
CLASS V. Rice.	5 to 8	Pentagonal or hexagonal, occasionally triangular. (Fig. 69.)	Angles sharply defined. Distinct hilum with a very high power, and cross visible in larger granules with polarised light.
Buckwheat.	5 to 20 depending on variety	Resembles oat and rice, but angles more rounded.	No rings, but distinct central hilum, as spot or star. Well-defined cross, with polarised light. Granules often compound.
Oat.	4 to 30	Triangular to hexagonal, a few small and round or apple-pip-shaped.	Rings and hilum invisible except under very high powers. Faint cross by polarised light.
Maize.	7 to 20	Circular to polyhedral, usually, with rounded angles. (Fig. 70.)	Hilum central, as a well-defined star or crack. Rings nearly invisible. Distinct cross and faint colours with polarised light.
Dari.	19	Small elongated hexagons	
Pepper.	½ to 5	Resembles rice, but majority decidedly smaller.	Shows hilum with very high power. Granules often in motion. Forms large compound granules of very irregular form.

Fig. 59.—Tous-les-mois starch × 240.   (*Greenish.*)

Fig. 60.—Potato starch × 240   (*Greenish.*)

Fig. 61.—Maranta starch × 240.
(*Greenish.*)

Fig. 62.—Curcuma starch × 240.
(*Greenish.*)

Fig. 63.—Wheat starch × 240.
(*Greenish.*)

Fig. 64.—Barley starch × 240.
(*Greenish.*)
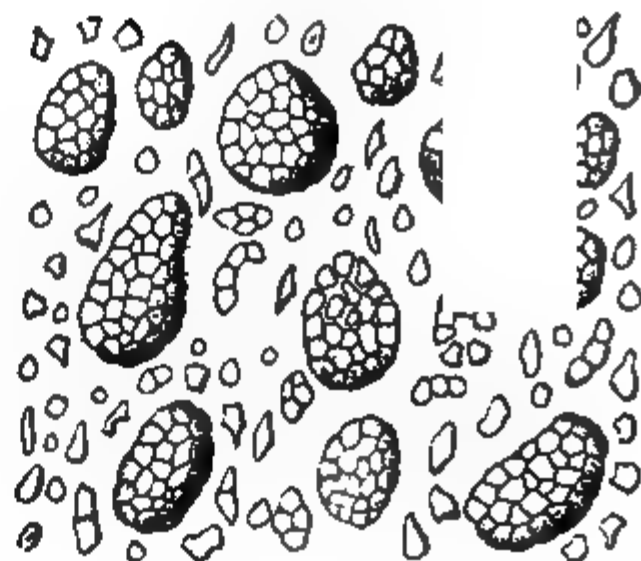
Fig. 65.—Rye starch × 240.
(*Greenish.*)

Fig. 66.—Oat starch × 240.
(*Greenish.*)

FIG. 67.—Sago starch × 240.
(*Greenish.*)

FIG. 67 a.—Sago × 240.
(*Greenish.*)

FIG. 68.—Tapioca starch × 240.
(*Greenish.*)

FIG. 68 a.—Tapioca × 240.
(*Greenish.*)
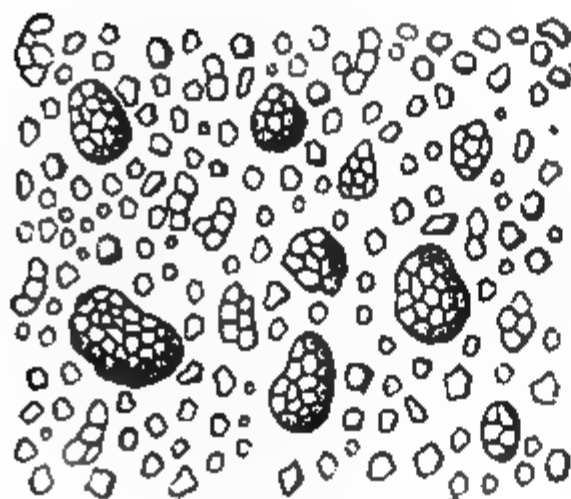
FIG 69.—Rice starch × 240
(*Greenish.*)

FIG 70.—Maize starch × 240.
(*Greenish.*)

Arrowroot of commerce is the starch derived from plants of the genus *Maranta*, belonging to the order *Marantaceæ*. For trade purposes arrowroot is distinguished by the name of the island or country producing it.

The starch corpuscles of the different species and varieties of *Maranta* differ considerably in their microscopic appearance, while certain varieties are closely simulated by the starches from plants other than the different species of *Maranta*. This is the case with the starch of *Curcuma angustifolia*, sometimes called East Indian arrowroot.

Arrowroot is liable to adulteration with a variety of cheaper starches, though the practice is now far less common than formerly. The principal starches which have been employed, either as substitutes for arrowroot or for mixing therewith, have been those of potato, sago, tapioca, curcuma, and tous-les-mois. Tacca and arum starches are also stated to have been employed, but they are not known at present in the English market.

The microscope affords the only satisfactory means of distinguishing maranta starch from the starches above mentioned, and even then the detection of certain admixtures is a matter of considerable difficulty. *Potato*[1] and *tous-les-mois* starches are distinguished by their large size and regular and well-developed concentric rings, and potato, in addition, by the hilum being situated near the smaller end of the granules. *Sago, tacca, arum* and *tapioca* are distinguished by the truncation of the granules. *Curcuma* starch forms oblong irregular granules rounded at the posterior extremity but which often taper rather abruptly at the other. The grains are so flat that when viewed on their edges they appear rod shaped.

The cereal starches may be divided into two well-defined groups, wheat, barley and rye starches being circular or nearly so, while the starches of rice, maize, buckwheat and oat are polygonal.

The leguminous starches present very close resemblances and are generally indistinguishable from each other when in admixture.

Proportion of Different Starches in Admixture.—The following method[2] is the most suitable, for ascertaining the extent to which oat-

[1] Besides its microscopical appearance, potato starch is said to be distinguished from maranta starch in the following respects: 1. When mixed with twice its weight of strong hydrochloric acid, maranta starch produces an opaque white paste, while the paste produced by potato starch is transparent and jelly like. 2. Potato starch evolves a peculiar and disagreeable odour when boiled with dilute sulphuric acid. 3. An acrid oil may be extracted from potato starch, but not from that of maranta.

[2] Dr. James Bell gives the following method for estimating starches in admixture: "The sample is first rubbed in a mortar and passed several times through a sieve. A small quantity, say 0.003 grm., is then weighed out and placed on a glass slide, where it is worked into a

meal is mixed with barley or wheat-flour, and is a type of the process to be employed in other cases. Genuine pearl-barley is ground finely in a mortar, and a series of standards made by mixing the flour with definite proportions of genuine oatmeal. Mixtures containing 5, 10, 15, 20, 30 and 40% of barley, respectively, will be found convenient in practice. The sample of oatmeal to be examined is thoroughly mixed, and 0.1 grm. weighed out and ground in an agate mortar with a little water. When the mixture is perfectly smooth it is rinsed into a small conical glass, and diluted with water to 10 c.c. Two of the standard mixtures (say the 10 and 20% mixtures) are then treated in a precisely similar manner. A drop of the sample and one of each of the standards are then placed on glass slides and covered with thin covers. Care must be taken that the starches and water are thoroughly agitated, so that the drops taken shall be representative, and it is important that the drops themselves shall be of exactly the same size. These conditions are best insured by immersing in the liquid a short piece of glass tube drawn out to a fine point, blowing down it so as to mix the sample thoroughly by means of the air-bubbles expelled, and then allowing a drop of the liquid to fall from the orifice on to the glass slide. The same tube is then employed to take drops of the standard mixtures. The cover-glasses must all be of equal size and sufficiently large to take up the whole of the drop, as none of the liquid must be removed. The slides being prepared, the number of barley granules visible in twelve successive fields is noted. The standards are then similarly observed, the operation being repeated until a standard mixture is found, the barley granules in twelve fields of which are equal or nearly equal in number to those counted in the sample. The proportion of wheat or barley in the sample will then be approximately the same as that in the standard it agrees with.

Detection and Estimation of Starch.—For the detection of starch existing in the *solid* state, no method is so good as the microscopic recognition of the corpuscles, the origin of which may usually be identified in the manner already described. The microscopic examination may be advantageously supplemented by adding a drop of iodine solution to the slide, when each of the true starch granules will assume

thin paste with about 2 drops of water. A thin covering glass, measuring about 1.5 in. by 1 in., is then placed over the paste, and moved about the slide until the paste is equally distributed and all under the covering glass. The number of granules is counted in nine fields, as fairly as possible representing the entire slide. The process is repeated till a correct idea of the composition of the sample is obtained. Standard mixtures approximately representing the sample are made up and treated in exactly the same way, and from a comparison of the results the percentage of foreign starch is computed."

a blue colour, which renders their recognition easy. In some cases, as when roasted coffee is mixed with beans or acorns, the microscopic detection of the starch becomes difficult, but may still be effected in the following manner: The coffee is boiled with water for a few minutes, and the solution is decanted or filtered from the insoluble matter. The liquid is next thoroughly cooled and cold dilute sulphuric acid is added. A solution of potassium permanganate is then gradually added till the brown colour is nearly or entirely destroyed, when the decolorised liquid is tested with iodine. A blue colour is obtainable in this way with coffee containing only 1 per cent. of roasted acorns.

Sometimes it is desirable to remove the colouring matter from the solid substance before examining it for starch. If cold water fail to effect this, alcohol should be tried, and subsequently other solvents. The cases are rare, however, in which the starch cannot be observed microscopically after successive treatments of the substance with cold water and alcohol.

In *aqueous solution*, starch yields a precipitate with ammoniacal lead acetate having a composition represented approximately by the the formula $C_{12}H_{12}Pb_2O_{11}$. Tannin gives a white precipitate with starch solution, disappearing on warming and reappearing as the liquid cools. Soluble starch is completely precipitated by adding alcohol to its aqueous solution.

The most characteristic reaction of starch solution is the violet or indigo-blue colouration which it gives with iodine. The coloured substance does not appear to be a definite compound of starch with iodine, a and hence is best called iodised starch. The best form in which to employ the reagent is as a very dilute solution of iodine in potassium iodide. The starch solution should be perfectly cold. On heating the liquid it is decolourised, but on cooling the blue is restored, though not with the same intensity as before. In employing the reaction as a test for starch it is necessary to remember that it is only produced by *free* iodine. Hence any free alkali should be neutralised by cautious addition of cold dilute acid, and any reducing or oxidising agent got rid of if possible. The best way of testing for starch is to add the iodine solution gradually to the slightly acid liquid until either a blue appears or the liquid remains permanently yellow by the free iodine. If the latter effect is produced and yet no blue is obtained no starch can be present.

The only organic compound liable to interfere when the test is per-

formed in the foregoing manner is erythrodextrin, which itself produces an intense reddish-brown colouration with iodine, which is apt to mask a feeble starch-reaction. The affinity of iodine for starch is, however, greater than its affinity for erythrodextrin, and hence if a very little iodine solution be employed the blue due to starch will alone be developed, the brown becoming apparent on a further addition of the reagent. By cautiously adding very dilute ammonia or gradually heating the liquid, the brown colour can be destroyed while the blue remains.[1]

Estimation of Starch.—The accurate estimatibn of starch is a difficult matter.

Chemical Methods.—These are based on the hydrolysis to reducing sugar by means of acids or enzymes and estimation of this either by means of its cupric reducing or optical powers or by fermentation to alcohol. In presence of vegetable tissue containing pentosans or other carbohydrates which yield reducing sugars on hydrolysis the acid method gives untrustworthy results, but it is applicable to the assay of commercial starches.

Hydrochloric Acid Method (as adopted by the A. O. A. C.).— 3 grm. of the material are extracted with 50 c.c. of cold water for an hour with frequent stirring. The residue is collected on a filter and washed with water sufficient to bring the filtrate up to 250 c.c. If the solution is difficult to filter, 2 c.c. of alumina cream are added. The soluble carbohydrates are determined in the filtrate both before and after inversion. The insoluble residue is heated for 2 1/2 hours with 200 c.c. of water and 20 c.c. of hydrochloric acid (sp. gr. 1.125) in a flask with a reflux condenser, cooled, nearly neutralised with sodium carbonate or sodium hydroxide made up to 250 c.c., filtered and the dextrose determined in an aliquot portion of the filtrate. The weight of dextrose multiplied by 0.9 gives the weight of starch.

Diastase Method.—This method which was originated by C. O'Sullivan in 1884 (*Trans.*, 1884, **45**, 1) is, on the score of accuracy, by far the best which has hitherto been proposed, but it is inconvenient owing to the length of time required to extract the amylans thoroughly.

The finely-divided grain is extracted with ether to remove fats, with alcohol to separate sugars and washed with water to remove amylans. The residue is transformed by diastase into maltose and dextrin, the

[1] Neither the brown colour of a solution of iodised erythrodextrin nor the blue of iodised starch shows absorption bands when examined by the spectroscope.

proportions of which are determined by Fehling's solution and by the polariscope. A fair sample of the grain is taken and 5.1 grm. weighed roughly and ground to a fine flour in a clean coffee-mill. 5 grm. of the powder are placed in a flask of about 120 c.c. capacity thoroughly wetted with rectified spirit, and 25 c.c. of ether added. The flask is corked and agitated occasionally, and after a few hours the ether is decanted through a filter and the residue washed by decantation with three or four fresh quantities of ether. To the residue 80 to 90 c.c. alcohol, sp. gr. 0.90, are added, and the mixture kept at 35° to 38° for a few hours with occasional shaking. The alcoholic solution, when clear, is decanted through the filter used in filtering the ethereal solution, and the residue washed a few times by decantation with alcohol of the strength and at the temperature indicated. The residue in the flask and any little that may have been decanted on to the filter, is then treated with about 500 c.c. of cold water. In about 24 hours the supernatant liquid becomes clear, when it can be gradually decanted through a filter. The solution filters bright, but, in the case of barley and oats, exceedingly slowly at times; the malted grains, as well as wheat, rye, maize, and rice, yield solutions requiring no excessive time to filter. The residue is repeatedly washed with water at 35° to 38°, but this treatment does not completely free barley and oats from a-amylan, which body dissolves with the greatest difficulty at this temperature. The residue is then transferred to a 100 c.c. beaker, and the portion adhering to the filter washed off by opening the filter-paper on a glass plate and removing every particle by means of a camel's-hair brush, cut short, and a fine-spouted wash-bottle. When the transference is completed, the beaker, which should not now contain more than 40 to 45 c.c. of the liquid, is heated to 100° for a few minutes in the water-bath, care being taken to stir well when the starch is gelatinising to prevent "balling" or unequal gelatinisation. After this the beaker is cooled to about 62°, and 0.025 to 0.035 grm. diastase,[1] dissolved in a few cubic centimetres of water, added.

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[1] The diastase employed is prepared as follows: Two or 3 kilogrm. of finely-ground pale barley-malt are taken, sufficient water added to completely saturate it, and when saturated to slightly cover it. When this mixture has stood three or four hours, as much of the solution as possible is pressed out by means of a filter-press. If the liquid is not bright it must be filtered. To the clear bright solution rectified spirit is added as long as a flocculent precipitate forms, the addition of the alcohol being discontinued as soon as the supernatant liquid becomes opalescent or milky. The precipitate is washed with alcohol of 0.86 to 0.88 sp. gr., dehydrated with absolute alcohol, pressed between cloth to free it as much as possible from that liquid, and dried *in vacuo* over sulphuric acid, until the weight becomes constant. Prepared in this way, the substance is a white, friable, easily soluble powder, retaining its activity for a considerable time. The preparation usually sold as diastase is useless for this work.

On keeping the liquid at 62° to 63° for a short time, the starch is completely converted into maltose and dextrin, and a drop of the solution no longer gives a blue colouration with iodine, but it is desirable to continue the treatment for about an hour after the disappearance of the starch, as the solution then filters more readily. The liquid is then heated to boiling for 10 minutes, and filtered, the residue being carefully washed with small quantities of boiling water. The filtrate is cooled, and made up to 100 c.c. and the density observed. The maltose is then determined by Fehling's solution, and the dextrin deduced from the rotatory power of the solution. The maltose found, divided by 1.055, gives the corresponding weight of starch, which, added to the dextrin found, gives the total number of grm. of starch represented by 100 c.c. of the solution.[1] The sum of the dextrin and maltose found directly ought to agree fairly well with the total solid matter estimated from the density of the solution, after making allowance for the weight of diastase employed.

The A. O. A. C. modifies this method as follows:

Extract 3 grm. of the finely powdered substance on a hardened filter with 5 successive portions of 10 c.c. of ether, wash with 150 c.c. of 10% alcohol, and then with a little strong alcohol. Place the residue in a beaker with 50 c.c. of water, immerse the beaker in a boiling water-bath, and stir the contents constantly for 15 minutes or until all of the starch is gelatinised; cool to 55°; add from 20 to 40 c.c. of malt extract and maintain at this temperature until a microscopic examination of the residue with iodine reveals no starch. Cool and make up directly to 250 c.c.; filter. Place 200 c.c. of the filtrate into a flask with 20 c.c. of 25 % hydrochloric acid (sp. gr. 1.125); connect with a reflux condenser and heat in a boiling water-bath for 2 1/2 hours; nearly neutralise while hot with sodium carbonate, and make up to 500 c.c. Mix the solution well, pour through a dry filter, and determine the dextrose in an aliquot part. Convert the dextrose into starch by the factor 0.90.

Preparation of Malt Extract.—Digest 10 grm. of fresh, finely-ground malt 2 or 3 hours at ordinary temperature, with 200 c.c. of

[1]In very accurate experiments it may be well to estimate the α-amylan present in the solution. For this purpose, 75 c.c. of the above solution should be evaporated to about 30 c.c., cooled, and 60 c.c. of rectified spirit added. A few drops of hydrochloric acid are then added, and the opalescent liquid stirred, when a flocculent precipitate will probably be produced. This is allowed to subside and the clear supernatant liquid is decanted off. The residue is then washed with alcohol of 0.86 sp. gr., dehydrated by treatment with strong alcohol, and collected on a tared filter. It is then dried *in vacuo* over sulphuric acid, and afterwards in dry air at 100° C., being subsequently weighed.

water, and filter. Ascertain the amount of dextrose in a given quantity of the filtrate after boiling with acid, etc., as in the starch determination, and make the proper correction.

The methods of estimating starch have been very fully investigated by Horace T. Brown (*Trans. Guiness Research Lab.*, 1903, 1, 79), who has made use of a modified Soxhlet extractor to facilitate the removal of the amylans in O'Sullivan's method.

An indirect method of determining starch in barley may be based on the fact that, if the products of starch hydrolysis with diastase are fermented with yeast in presence of a small quantity of active diastase, the dextrins as well as the maltose can be completely fermented.

5 grm. of barley are extracted with ether and alcohol (sp. gr. 0.90); the residue is washed into a flask and thoroughly boiled to remove alcohol and to gelatinise the starch and converted with 6 c.c. of an active malt extract and fermented without previous destruction of the diastase with 1 grm. of yeast at 26–27°, for several days. The alcohol is determined by the distillation method; a control solution with yeast, malt extract and water is kept under identical conditions and the alcohol produced allowed for. 92 parts of alcohol represent 153.9 parts of starch.

Horace Brown's rapid method enables the estimation of starch in a barley to be carried out in 5 hours.

5 grm of the grain are extracted in a suitable apparatus with alcohol (sp. gr. 0.90) for 3 hours, transferred to a beaker containing 100 c.c. of water and the whole thoroughly boiled after cooling to 57°, 10 c.c. of an active malt extract are added and the conversion allowed to proceed for 60 minutes. The solution is boiled, filtered into a 200 c.c. flask, the residue well washed and the volume adjusted after cooling. The cupric reduction of 20 c.c. is determined and the maltose calculated from the copper reduced after the correction for the reduction due to the malt extract. 84.4 parts of maltose correspond to 100 parts of starch. The malt used should have a diastatic power of 80 Lintner.

Märcker and Morgen's method, much used on the Continent, is as follows: 3 grm. of finely divided, fat free substance are heated in a small metal vessel with 50 c.c. of water for 20 minutes at 100°, cooled to 79°, and liquefied by means of 5 c.c. of malt extract for 20 minutes. 5 c.c. of 1% tartaric acid are added and the vessel heated in a Soxhlet digester for half an hour at a pressure of 3 atmospheres.

Following a further addition of 5 c.c. of tartaric acid, the vessel is removed and again heated for 20 minutes at 70°. The solution is filtered, diluted to 200 c.c. and inverted by boiling with 15 c.c. of hydrochloric acid (sp. gr. 1.125) for 3 hours in a flask attached to a reflux condenser. The liquid is cooled, neutralised by sodium hydroxide, made up to 500 c.c. and the sugar estimated by Fehling's solution.

Polarimetric Estimation of Starch in Cereals.—C. J. Lintner (*Z. ges. Brauw.*, 1907, **30**, 109) gives the following method: 5 grm. of the very finely powdered cereal are triturated in a mortar with 20 c.c. of water until no lumps remain; 40 c.c. of concentrated hydrochloric acid are then added and the mixture left for 30 minutes; the pale yellow-coloured paste will have become darker and more fluid. The liquid is then washed into a measuring flask of 200 c.c. capacity by means of hydrochloric acid of sp. gr. 1.125, 10 c.c. of 4 % solution of phosphotungstic acid are added to precipitate the proteins, and the volume made up to 200 c.c. with the diluted hydrochloric acid. The liquid is shaken and filtered and the clear filtrate examined by the polarimeter in a 2 dcm. tube. The concentration of the soluble starch is calculated on the basis of $[a]_D = 200.3°$ for barley starch dissolved in hydrochloric acid at 20°. Provided the liquid be not allowed to remain longer than 2 hours before polarising, no decrease in the rotatory power need be feared. The method gives results 4 to 6% lower than the acid inversion process, owing to the pentosans, etc., being counted as starch in the latter process.

Canet and Durieux (*Bull. Soc. Chim. Belg.*, 1907, **21**, 329) find Lintner's method to be generally applicable. They use Brown's figure $[a]_D = 202°$ for the specific rotatory power of starch. Unless the material is rich in nitrogenous matter, it is not necessary to add phosphotungstic acid. The material should be free from fat and be dried before treatment. A few of the results may be cited; they are expressed as dry starch on air-dry material.

Potato fecula 84.15; maize starch 82.66; rice starch 83.06; corn flour 70.04; rice flour 76.73; malt flour 50.02; yellow maize 58.16; maize grits 75.43 ; bran 23.51; brewers' grains 1.73 to 9.10; maize press cake 8.66.

Wenglein (*Z. ges. Brauw.*, 1908, **31**, 53) substitutes sulphuric acid of sp. gr. 1.70 for hydrochloric acid in Lintner's process and washes with acid of sp. gr. 1.30, but the method is otherwise the same. The

solutions in sulphuric acid can be kept 8 hours unchanged before polar-ising $[a]_D = 191.7°$ for starch in sulphuric-acid solution.

The above authors consider the optical activity of other constituents in starch-yielding cereals to be so small as to be negligible. Ewers (*Z. öffentl. Chem.*, 1908, **14,** 8) disputes this and states that a con-trol experiment to determine the optical rotation of the soluble carbo-hydrates is always necessary. For this control 5 grm. of the substance and 70 c.c. of water are digested for 1 hour at 50°, then 25 c.c. of glacial acetic acid are added and the digestion continued for half an hour. The filtered extract is made up to volume and polarised.

Gschwendner (*Chem. Zeit.*, 1906, **30,** 761) advises the following method for the valuation of maize and cereals: 5 to 7.5 grm. of meal are shaken with 25 to 30 c.c. of acidified brine (made by dissolv-ing 100 grm. of salt in 400 c.c. of water and adding 50 c.c. of 23% hydrochloric acid) in a flask attached to a reflux condenser and heated in a calcium chloride bath for 1 1/4 hours. The contents are clarified with 5 c.c. basic lead acetate, diluted to 50 c.c., an excess of water added corresponding to the volume of the undissolved residue added, and the filtrate polarised. The rotatory power is calculated as dextrose and multiplied by 0.9 to give the starch.

Parow and Neumann (*Z. Spiritusind.*, 1907, **30,** 561) modify this method somewhat and find it gives satisfactory results.

Commercial Starch.—This is usually obtained from wheat, rice, maize or potatoes. In England hardly any starch is now made from wheat. Characteristic of wheat starch is the coherence of the granules due to the small admixture of gluten. A rough estimation of the starch in wheat flour may be effected by washing a weighed quantity over a muslin sieve in a stream of water. The gluten remains and the water containing the starch is allowed to stand until the starch has settled, when it is collected, dried at 110° and weighed.

The ash of starch is trifling in amount, its estimation serves to detect any mineral additions.

The moisture may be determined by drying in a vacuum over sul-phuric acid or in a current of dry air at 100°. For approximately estimating the water in potato starch, Saare's method is more con-venient. It consists in placing 100 grm. of the sample in a 250 c.c. flask, filling the flask to the mark with water at 17.5°, and observing the weight of the contents. There is no occasion to employ the large quantities of starch and water recommended by Saare. He gives a

table (*Jour. Soc. Chem. Ind.*, 1884, **3**, 527) by which the proportion of water is directly shown, but the following rule may be employed instead: From the weight of the starch and water contained in the bottle subtract 250, and divide the difference by 0.3987, when the quotient will be the percentage of starch in the sample. This instruction applies to the quantities of starch and water prescribed by Saare, but the following is a more general expression of the rule:

$$\frac{\text{Contents of bottle in grm. } \textit{minus} \text{ capacity of bottle in c.c.}}{0.3987} = \begin{cases} \text{grm. of anhydrous starch in} \\ \text{weight of sample taken.} \end{cases}$$

The method gives values within 0.5% and an estimation can be made in 30 minutes. J. H. Hoffmann (*Woch. für Brauerei.*, 1903, No. 31) heats the starchy matter to drive out the water which is condensed, collected and measured. 50 grm. of starch are immersed in 400 c.c. of oil of turpentine and 10 c.c. of toluene in a boiling vessel and heated first at 50°, then to 140° and finally to 155° for 5 minutes in each case. The water formed is collected and measured, a correction of 0.2 c.c. added and the whole multiplied by 2 to give the percentage of water in the starch.

For technical purposes it is sometimes desired to estimate the proportion of starch existing in potatoes. This can be done in a rough and ready manner by ascertaining the sp. gr. of the tuber. The unpeeled potatoes, freed from dirt, are placed in a solution of salt, which is then diluted with water till some of the individual tubers sink, while others just float. The density of the saline solution, as ascertained by a hydrometer, is then equal to the average sp. gr. of the potatoes. Another method consists in taking 5 kilogrm. of the potatoes, and then weighing in water. The weight in water divided into the original weight in air gives the sp. gr. Tables have been compiled for ascertaining the percentage of starch from the sp. gr. of the potatoes.

The sp. gr. ranges from 1.08 to 1.15, the heaviest potatoes containing most starch and most dry matter. The most used tables are those of Behrend, Märcker and Morgen (*Zeit. für Spiritusind.*, 1879, 361), which give results accurate within 2%. An approximate estimation may be based on the following: A potato containing 19.9% of dry matter has a sp. gr. 1.081; an increase of 0.001 in the sp. gr. corresponds to 0.214% of dry matter. The dry matter less 5.75 is equal to the weight of starch contained.

Starches are often graded according to the stiffness of the pastes they yield under comparable conditions when boiled with water and

cooled. Ermen (*J. Soc. Chem. Ind.*, 1907, 26, 501-502) makes solutions of starch in the cold with the help of sodium hydroxide and determines their viscosities in a Redwood viscometer.

The weighed sample of starch is shaken continuously with 230 c.c. of cold water and 15 c.c. of a 10% solution of sodium hydroxide with the addition of enough water to bring the whole up to 250 c.c. until the solution begins to thicken. It is allowed to stand until the next morning before measurement. When close attention is paid to constancy of procedure, the method is claimed to give concordant results with the same starch whilst different starches and different brands of the same starch are easily differentiated.

DEXTRIN. AMYLIN.

Dextrin is a product obtained by treating potato, wheat or maize starch or other amylaceous bodies in certain ways. The following modes of treatment cause a formation of dextrin:

By heating starch or flour at a temperature ranging from 210° to 280° until it acquires a yellow or brownish colour. The change is greatly facilitated by moistening the starch with dilute nitric or other acids, and then slowly drying the paste and heating it for some time to about 110° to 150°.

By boiling starch with dilute sulphuric acid till the cooled liquid no longer gives any colouration with solution of iodine.

By treating gelatinised starch with warm water and a small quantity of malt extract.

The first process is employed for the manufacture of solid dextrin, which is known in commerce by the name of British gum, gommeline, starch-gum, etc. The other processes result in a simultaneous formation of maltose, as described elsewhere. The former is used for the preparation of commercial glucose, and the latter reaction takes place in mashing malt for the manufacture of beer.

Several, and not impossibly many, varieties of dextrin exist, all being apparently formed by the breaking up of the highly complex starch molecule by treatment with dilute acids or ferments. There is no ready method of distinguishing the different varieties with certainty, except that one kind, or possibly class, of dextrin gives a reddish-brown colour with solution of iodine, while the other kind or class produces no colouration. The erythrodextrin, the kind giving the brown colour

with iodine, is an intermediate product of the formation of achro-dextrin from starch.

The best method of applying the iodine reaction as a test for erythro-dextrin is to divide a very weak solution of the iodine in potassium iodide into two parts, and place the slightly yellow liquid in adjacent test-tubes or glass cylinders. On then adding the solution to be tested to one, and an equal measure of water to the other, any brownish colouration will be readily observed. In presence of starch, the blue is apt to obscure the brown tint produced by the erythrodextrin. This may be avoided to some extent by employing the iodine solution somewhat in excess, so as to get a full development of the brown.

Pure dextrin is a white amorphous solid. It is tasteless, odourless, non-volatile and very deliquescent. It dissolves in an equal weight of cold water to form a syrupy, dextro-rotatory, liquid, $[a]_D = 200°$, which is miscible with 1.5 measures of proof spirit. By strong spirit, if used in sufficient quantity, dextrin is completely separated from its aqueous solutions.

Cold concentrated sulphuric acid dissolves dry dextrin without colour, but charring takes place on warming. By boiling with dilute acids, dextrin yields maltose and ultimately dextrose. Hot nitric acid of 1.35 sp. gr. converts dextrin in part into oxalic acid, whereas the natural gums yield mucic acid under similar conditions.

Dextrin is distinguished from starch by its solubility in cold water; from soluble starch by yielding no blue with iodine when tested as described on page 419, and no precipitate with baryta water; from maltose and dextrose by not reducing Fehling's solution; from starch, soluble starch, gelatin and egg-albumin by not yielding a precipitate with tannin; from albumin by not being coagulated by heat or mineral acid.

Dextrin is separated from starch and cellulose by solution in cold water; coagulable proteins may then be separated by raising the faintly acid solution to boiling. An ammoniacal solution of lead acetate added to the cold and dilute liquid is stated to precipitate the dextrin, leaving the sugar in solution. The precipitate may be dried at 100°, and is said to have the formula $PbO,C_6H_{10}O_5$. Another method consists in precipitating the dextrin by means of a large proportion of alcohol, washing the precipitate with rectified spirit, and drying it at 110°. After weighing, the dextrin should be ignited, and the resultant ash deducted from the total weight obtained.

The proportion of dextrin present in a solution also containing maltose and dextrose may be determined by observing the rotatory action of the liquid, together with its sp. gr. and reducing action on Fehling's solution.

Commercial Dextrin.—Commercial dextrin, or "British gum," is now manufactured extensively by moistening starch or flour with a mixture of dilute nitric and hydrochloric acids, and then exposing it to a temperature of 100° to 125°. Either nitric or hydrochloric acid singly may be substituted for the mixture or oxalic acid may be employed.

Commercial dextrin is a white, yellowish or light brown powder. It consists largely of erythrodextrin, and hence its aqueous solution is coloured brown with iodine, unless this reaction is obscured by the blue produced by a considerable proportion of soluble *starch*. For most purposes this admixture is unobjectionable, provided that it does not exceed 12 or 15 %. *Unaltered starch* may be recognised by the microscope and its insolubility in cold water. Reducing *sugars* (maltose) are nearly always present in commercial dextrin, and may be detected and estimated by Fehling's solution.

Many mixtures of starch and dextrin are employed as thickening agents in calico-printing, etc. "Gloy" consists essentially of farina mixed with a solution of magnesium chloride.

Dextrin syrups are largely employed by confectioners. Their examination is described under "glucose."

The method of distinguishing commercial dextrin from gum arabic is described on page 442.

CELLULOSE,[1] $C_6H_{10}O_5$.

Cellulose constitutes the essential part of the solid frame-work or cellular tissue of plants and is an especially characteristic product of the vegetable kingdom. Tunicin, a compound resembling cotton cellulose in many ways, is obtained from the outer coating of *Ascidia* and other invertebrate species.

Cellulose occurs nearly pure in cotton, linen and the pith of certain plants. Still purer forms are Swedish filter-paper, linen rags and cotton wool. Cellulose is more stable than starch. It is insoluble in water and all simple solvents. Air-dry cellulose contains from 6 to 12 %

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[1] For full information on the subject of cellulose the work of Cross and Bevan on *Cellulose* should be consulted, also "*Researches on Cellulose*," 1895–1900, 1900–1905.

of water which is readily driven off at 100°, but reabsorbed on exposure to the atmosphere, and is termed "moisture of condition." This moisture is of much importance in the processes of spinning and finishing fibres and also in the buying and selling of fibrous products.

Cellulose hydrates give an indigo-blue colouration with iodine in aqueous solution. They do not, however, differ in their essential properties from cellulose.

The water of hydration of a cellulose may be determined as the difference between the hygroscopic moisture lost at 100–105°, and that which is driven off at the temperature of boiling toluene. The sample is boiled with toluene and the water which distils over is absorbed by calcium chloride and weighed after removal of the hydrocarbon.

To determine the degree of hydration of a cellulose, the sample is stained blue with zinc chloride-iodide reagent and the rapidity with which the colour is removed by water is noted. Highly hydrated celluloses retain the colour for a considerable time.

Solvents of Cellulose.—1. Aqueous zinc chloride: 4 to 5 parts of zinc chloride are dissolved in 5 to 7 parts of water and 1 part of cotton cellulose stirred in till evenly moistened, the solution being heated in a porcelain dish over the water-bath. It is stirred from time to time and water added to replace that which evaporates. The solution forms a precipitate of cellulose hydrate containing zinc salt on pouring into water or alcohol. Digestion with dilute hydrochloric acid removes the zinc salt. This solution is employed for making cellulose threads which are carbonised for use in the incandescent electric lamp. Zinc chloride dissolved in twice its weight of 40% aqueous hydrochloric acid dissolves cellulose rapidly in the cold, but the cellulose undergoes a gradual hydrolysis.

2. Ammoniacal cupric oxide (Schweitzer's reagent) contains 10 to 15% ammonium hydroxide and 2 to 2.5% copper as CuO. It is prepared either by adding ammonium chloride and an excess of sodium hydroxide to a solution of cupric salt and redissolving the well-washed blue precipitate in ammonia (0.92 sp. gr.) or by immersing copper turnings in strong ammonia in a cylinder and bubbling air or oxygen through the liquid for about 6 hours. On treatment with the cuprammonium solution, cellulose becomes gelatinous and on agitation gradually dissolves forming a viscid solution which may be filtered after dilution with water.

On neutralising the filtrate with hydrochloric acid the cellulose is separated in a flocculent state resembling aluminum hydroxide, which when dried forms a brittle, greyish, horn-like mass. Carbon dioxide also precipitates the solution, as do sugar, salt and even copious dilution with water.

The solution of cellulose in Schweitzer's reagent is decomposed by dialysis. It is lævorotatory, a 1% solution showing a specific rotation of —20° for the light transmitted, which bears to the sodium ray the ratio 1:1.85. The optical activity is not strictly proportional to the cellulose dissolved, increasing somewhat more slowly than the concentration of the solution.

Fabrics passed through a bath of the reagent are surfaced by the film of gelatinised cellulose and compacted together so that the fabric becomes waterproof. The cellulose retains the copper hydroxide which acts as a preservative.

On prolonged boiling with dilute acids, cellulose is converted into hydrocellulose which differs from cellulose in containing free carbonyl groups and in the greater reactivity of its hydroxyl groups. Cold concentrated sulphuric acid rapidly attacks and dissolves cellulose with the formation of dextrin-like bodies. If the solution be now diluted and boiled, dextrose is formed as the chief product of hydrolysis. Hot concentrated sulphuric acid at once chars cellulose. On treatment with sulphuric acid diluted with half its volume of water (sp. gr. 1.5 to 1.6) cellulose is gelatinised and converted into a substance termed amyloid, which is coloured blue by iodine. Paper placed in an acid of this strength for a short time and then transferred to water has a tough coating of amyloid fixed on its surface and constitutes parchment paper.

By treatment with cold nitric acid of 1.42 sp. gr. cellulose is remarkably toughened, without losing its fibrous structure or becoming nitrated. With stronger acid, cellulose is converted into nitro-substitution products which are described elsewhere.

Nitric acid (sp. gr. 1.1 to 1.3) oxidises cellulose to oxycellulose; dilute chromic acid has a similar effect. Hypochlorites in dilute solution (1%) have only a very slight action on cellulose proper. Permanganates in neutral solution also attack cellulose, but slowly. In stronger solutions, the fibre substance is oxidised and disintegrated and an oxycellulose results. The joint action of hypochlorite solutions and carbonic acid rapidly produce oxycellulose, which acquires

the property of selective attraction for certain colouring matters—
notably the basic coal-tar dyes. Oxycelluloses in overbleached cloth
may thus be easily detected by immersion of the fabric in a dilute solu-
tion of methylene blue.

Cellulose is very resistant to dilute alkaline solutions even at high
temperatures. Cellulose fibres are freed by drastic treatment with 1 to
2 % sodium hydroxide from non-cellulose constituents which become
saponified. Cold solutions containing above 13 % sodium hydroxide
cause a remarkable change in the structure of the fibre which, seen in
the mass, causes a shrinkage in length and width with an increase
in thickness. The compound of cellulose and alkali formed is de-
composed on washing with water, the cellulose reappearing as the
hydrate $C_{12}H_{20}O_{10}$. This is known as mercerised cellulose.

This alkali-cellulose reacts with carbon disulphide forming an
alkali-cellulose-xanthate which is perfectly soluble in water, giving
a very viscous solution which is precipitated in the form of a gelat-
inous hydrate by various neutral dehydrating liquids or solutions.
To prepare it, cellulose is treated with excess of 15% solution of
sodium hydroxide and after standing for some time separated from
the liquid, squeezed to remove excess and mixed with 40 to 100 parts
of carbon disulphide. After some hours, the yellowish mass is covered
with water and subsequently stirred with more water when solution
occurs.

Cellulose is regenerated from this solution on standing some days or
on heating at 80 to 90°. It has 3 to 4% more moisture and cor-
responds to the formula $4C_6H_{10}O_5.H_2O$. In general it is far more
reactive than the original cellulose.

If cotton-wool or filter-paper be heated at 180° for several hours
with about 6 or 8 parts of acetic anhydride, it is entirely dissolved
and converted into a triacetate, $C_6H_7(C_2H_3O)_3O_5$, which may be sepa-
rated by pouring the syrup into water; it is a white powder, optically
inactive, soluble in strong acetic or sulphuric acid, and very readily
converted into cellulose and potassium acetate by boiling with dilute
caustic potash. Other acetyl-derivatives of cellulose have been
obtained.

The estimation of the cupric reducing power is said to afford
a useful measure of the free carbonyl groups in cellulose and hence
of the chemical condition of the sample. Schwalbe (*Ber.*, 1907, **40,**
1347 to 1351) determines this in the following manner:

Two portions, of about 3 grm. each, of the cellulose are weighed out; one portion serves for the determination of the absolute dry substance, whilst the other is reduced to a fine state of division, without drying by heat, and is mixed with 200 c.c. of water and 100 c.c. of Fehling's solution. The liquid is boiled for 15 minutes under a reflux condenser, with frequent agitation. The liquid is then filtered hot and the residue containing the cuprous oxide is then dissolved in nitric acid and the amount of copper determined, preferably by the electrolytic method. The "copper value" represents the percentage of metallic copper calculated on the dry cellulose. Cotton wool has a value about 1.7, bleached sulphite wood pulp about 3.9, overbleached sulphite wood pulp 19.3.

In a second paper (*Ber.*, 1907, **40**, 4523–4527) Schwalbe applies the test to artificial silks. Viscose and Pauly silks, both made by alkali processes, have low copper values, about 0.8. Chardonnet silk, being made by an acid process, has a value of 3.1.

Hydrocelluloses show values of 2 to 8.8 according to the degree of hydrolysis. Oxycelluloses have much higher copper values, 7.6 to 35.

Plant celluloses may be classified in 3 groups (Cross and Bevan):

1. Those of maximum resistance to hydrolysis which contain no carbonyl groups—normal cellulose.

2. Those of less resistance to hydrolysis and containing active carbonyl groups—the oxycelluloses.

3. Those easily hydrolysed by acid or in some cases by enzymes to form simple carbohydrates and which are more or less soluble in alkaline solutions—the pseudo- or hemi-celluloses.

To establish the nature of a cellulose, it is generally sufficient to determine the ultimate composition, resistance to alkaline hydrolysis, behaviour with solvents, reaction with sulphuric acid (solution without blackening) and with a nitrating mixture (nitric acid and sulphuric acid).

Materials composed of normal and resistant celluloses only are quite inert and have lasting properties. Those containing oxidised and oxycelluloses, also lignocelluloses, are liable to discolouration and are far more perishable.

The **lignocelluloses**, of which the jute fibre is a typical representative, differ markedly from the celluloses. They have a higher $\dfrac{CH}{O}$ ratio,

contain unsaturated groups which combine with chlorine to form quinone bodies. They contain a furfural yielding complex, methoxyl groups and an acetic acid residue. These constituents make up the non-cellulose part of the fibre often termed lignin, in addition to which it contains cellulose proper which can only be isolated after the chemical decomposition of the non-cellulose.

Lignocelluloses possess a number of characteristic reactions. Salts of aniline colour the fibre a deep golden-yellow. Phloroglucinol, dissolved in hydrochloric acid, gives a deep magenta colouration. Iodine is absorbed in large quantity, colouring the fibre a deep brown. The fibre readily combines with chlorine, as shown by the characteristic magenta colouration developed on the subsequent addition of sodium sulphite. Very characteristic is the reaction with ferric ferricyanide obtained by mixing equivalent proportions of potassium ferricyanide and ferric chloride. The fibre stains a deep blue and takes up a considerable quantity of pigment.

Pectocelluloses.—These contain a larger proportion of oxygen than the celluloses and give a series of pectic acids and insoluble cellulose on hydrolysis with dilute alkaline solutions; they are saturated compounds.

Pectose occurs in the utricular tissue of fruits and roots. It is insoluble in water, but is converted into soluble pectin on hydrolysis with dilute acids or alkalies or by an enzyme, *pectase*. In addition to the pectocelluloses proper, as typified by flax, esparto, etc., are the *mucocelluloses;* these are decomposed by the action of water, forming the solutions known as mucilages, which are neutral and on ultimate hydrolysis give rise to the formation of various hexoses and pentoses.

The cutocelluloses are associated with oily and waxy products which add to their water-resisting property, but are removed by solvents. On decomposition by oxidation and saponification, a large additional quantity of such products is formed.

Cutose, or cuticular substance, constitutes the greater part of cork, and the fine transparent membrane covering the exposed parts of vegetables. It contains a high percentage of carbon (C=68.29; H=8.95), and yields suberic acid, $C_8H_{14}O_4$, on oxidation with nitric acid of 1.20 sp. gr. Cutose is insoluble in cold sulphuric acid of 1.78 sp. gr. and in the cuprammonium solution which dissolves cellulose. On the other hand, it dissolves slowly in a hot dilute solution of sodium hyoxide or carbonate, forming a solution from which acids

precipitate a yellowish, flocculent substance, fusible below 100°, soluble in alcohol and ether, and having the same composition as cutose. If the alkaline solution be saturated with common salt, a cutose-soap rises to the surface. From the researches of Urbain, cutose appears to be composed of stearocutic acid, $C_{28}H_{48}O_4$, with 5 equivalents of oleocutic acid, $C_{14}H_{26}O_4$.

Purification of Cellulose in the Laboratory.[1]—1. The fibre is first subjected to alkaline hydrolysis, *i. e.*, boiling with dilute sodium hydroxide, carbonate or sulphite.

2. It is then exposed to chlorine gas or bromine water or oxidised by means of hypochlorites or permanganates. The use of the last necessitates treatment with sulphurous acid to remove manganese dioxide deposited on the fibre.

3. Finally process 1 is repeated to remove products rendered soluble by process 2.

In consequence of its occurrence in association with bodies of a closely allied nature, the accurate determination of cellulose is often a tedious operation, and some, at least, of the processes prescribed for the purpose yield arbitrary rather than accurate results.

From *starch* cellulose is best separated by boiling the substance with water containing 1% by volume of sulphuric acid. The liquid is filtered when a drop taken out gives no colouration with iodine solution. In cases in which the use of acid is objectionable, the sub-substance should be boiled with water, and the unfiltered liquid mixed with an equal measure of cold infusion of malt. The starch will be wholly dissolved by keeping the liquid at a temperature of 60° for a short time. The separation of cellulose from *sugar, dextrin* and other substances soluble in water presents no difficulty. Proteins may be separated by treatment with warm water containing 1% of alkali. They may be determined by the methods for nitrogen.

For the estimation of cellulose in wood, vegetable fibres and substances to be used for the manufacture of paper, Müller recommends the following process: 5 grm. of the finely-divided substance are boiled 4 or 5 times with water, using 100 c.c. each time. The residue is dried at 100°, weighed, and exhausted with a mixture of equal measures of benzene and strong alcohol, to remove fat, wax, resin, etc. The residue is again dried, and boiled several times with water to every

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[1] In estimating fibrous precipitates it is often advisable to collect on an asbestos filter, dry at 110° and weigh, after which the filter and its contents are ignited and again weighed. The loss in weight gives the amount of precipitate.

100 c.c. of which 1 c.c. of strong ammonia has been added. This treatment removes colouring matter and pectous substances. The residue is further bruised in a mortar, if necessary, and is then treated in a closed bottle with 250 c.c. of water and 20 c.c. of bromine water containing 4 c.c. of bromine in 1000 c.c. In the case of the purer bark-fibres, such as flax and hemp, the yellow colour of the liquid only slowly disappears, but with straw and woods decolourisation occurs in a few minutes. When this takes place, more bromine water is added, and this repeated till the yellow colour remains and bromine can be detected in the liquid after twelve hours. The liquid is then filtered, and the residue washed with water and heated to boiling with 1000 c.c. of water containing 5 c.c. of strong ammonia. The liquid and tissue are usually coloured brown by this treatment. The undissolved matter is filtered off, washed, and again treated with bromine water. When the action seems complete, the residue is again heated with ammoniacal water. This second treatment is sufcient with the purer fibres, but the operation must be repeated as often as the residue imparts a brownish tint to the alkaline liquid. The cellulose is thus obtained as a pure white substance. It is washed with water, and then with boiling alcohol, after which treatment it may be dried at 100° and weighed.

Maximum yields of cellulose are obtained by the chlorination process (Cross and Bevan, *Trans. Chem. Soc.*, 1889, **55**, 199): 5 grm. of the fibre dried at 100° are boiled for 30 minutes with 1% sodium hydroxide, well washed on a gauze filter, squeezed to remove excess of water and placed in a beaker into which a slow stream of washed chlorine gas is passed. The fibre changes in colour from brown to golden-yellow; after 30 to 60 minutes' exposure it is removed, washed and heated in a 2% solution of sodium sulphite to boiling when 0.2% sodium hydroxide is added and boiling continued for 5 minutes. The cellulose is then filtered and washed and is almost white; it may be finally bleached by immersion in dilute hypochlorite or permanganate solution (0.1%). The amount of cellulose is 2 to 5% higher than that yielded by Müller's process and the method far less tedious.

Other methods involve the prolonged digestion with nitric acid and potassium chlorate or with dilute nitric acid at 50 to 80°, but are of subordinate interest.

Lignocelluloses give a blood-red tint with a reagent prepared by

dissolving 2 grm. of paranitraniline in 100 c.c. of hydrochloric acid (sp. gr. 1.06) especially on heating.

The amount of furfural yielded by fibres on boiling with hydrochloric acid is estimated by Tollens' method as described under Pentoses.

Methoxyl is estimated by boiling the fibre substance with concentrated hydriodic acid (*Zeisel's method*).

To determine cellulose, lignin and cutin in crude fibre, König (*Z. Unters. Nahr. u. Genussm.*, 1906, **12**, 385–395) digests the fibre in the cold with hydrogen peroxide in presence of ammonium hydroxide, the treatment being continued for a long time with successive additions of hydrogen dioxide until the residue is colourless. The treatment oxidises the lignin and converts it into soluble products. The residue, consisting of cellulose and cutin, is treated with the cuprammonium solvent to dissolve the cellulose, the cutin remaining unattacked. The liquid is filtered on a gooch asbestos filter and the cutin residue weighed, the cellulose is precipitated by alcohol and weighed and the weight of crude fibre less the weight of these two is taken as lignin.

König states from the investigations of hay and bran that lignin contains 55.3 to 59.0% of carbon and cutin 60 to 75.4% of carbon. Cellulose from the same source contains methoxyl groups varying in proportion from 0.4 to 2.82%. Similar methoxyl groups were unfound by Cross, Bevan and Beadle in the cellulose from jute. The lignin contains not only methoxyl groups, but also ethoxyl and acetyl residues.

Estimation of Crude Fibre.—In valuing food stuffs a distinction is made between the digestible and indigestible constituents. As the process of animal digestion is in reality an exhaustive series of alternately acid and alkaline hydrolyses, a standard method of estimating the crude fibre has been adopted, consisting in boiling the material first with sulphuric acid and then with sodium hydroxide. For the A. O. A. C. process see page 70.

Agar-Agar

Agar, often called Japanese isinglass, is a colloid substance prepared from marine algæ. It consists of a carbohydrate, sometimes called gelose, which is a polymer of galactose and is converted into galactose on boiling with dilute mineral acids. Tollens uses the more correct term d-galactan for gelose. It has the formula $(C_6H_{10}O_5,)_n$ is lævorotatory at first in warm aqueous solution, but changes to dextro-

rotatory on prolonged warming.   Agar also yields a small proportion of pentoses when hydrolysed.

It occurs in transparent strips of the thickness of a straw or in shorter and thicker yellowish white pieces.   It is odourless and tasteless, insoluble in cold water, soluble in hot water.   On cooling, the solution gelatinises to a thick jelly that does not melt as readily as that from gelatin.   The solution in 500 parts of water still gelatinises when cold. It is chiefly employed as a culture medium for bacteria and also used as a thickening agent in milk and cream and as a substitute for white of egg.

The aqueous solution should give no precipitate with tannic acid solution, proving the absence of gelatin, and no blue colouration with iodine, proving the absence of starch.

Commercial agar usually contains diatoms, a characteristic form being *Arachnoidiscus Ehrenbergii* (see Fig. 71).   To obtain the diatoms, the organic material is oxidised with a mixture of nitric and sulphuric acids.

FIG. 71.—*Arachnoidiscus Ehrenbergii.*   × 100.   The smaller oval diatoms are a species of *Cocconeis.*   (*Leffmann.*)

Agar is much used for thickening cheap ice-cream, especially that sold in American cities under the name "Hokey-Pokey."   It is adapted for such use because its jelly does not liquefy at so low a temperature as that made with gelatin.   Agar has also been offered as a substitute for gelatin in ordinary diet because, being practically indigestible and not irritating, it is supposed to give a bulkiness to the contents of the intestine and promote the peristaltic movements.

GUMS.

Gums are a peculiar class of bodies occurring in the juices of plants. They are perfectly non-volatile, have little or no taste, are uncrystallisable, and eminently colloidal.   These characters render their pur.-

fication very difficult, and hence but little s known of their chemical relationships. For convenience, various pectous bodies are classed with the gums.

The analytical characters of the gums as a class are indicated by the following facts, which are also applied to their separation from similar bodies.

Gums are either soluble in, or swell up in contact with, cold water, a character which distinguishes them from starch, cellulose and resins. They differ from the sugars by being incapable of fermentation by yeast, and from the sugars and resins by their insolubility in alcohol. From dextrin the gums soluble in water are distinguished by their lævorotatory power and acid reaction, and by yielding mucic acid on treatment with moderately concentrated nitric acid. Reichl and Breinl state that arabin and bassorin are distinguishable from dextrin by the blue flocculent mass they yield when heated with hydrochloric acid and orcinol, dissolving in alcoholic potash to form a violet solution showing a green fluorescence. Fragments of wood, containing only traces of wood-gum, when boiled with hydrochloric acid and orcinol show the reaction quite distinctly. From erythrodextrin and starch the gums differ by giving no colour with solution of iodine, and from proteins they are distinguished by not yielding ammonia when ignited with soda-lime.

The gums of which gum arabic is the type are dissolved by cold water and are not readily precipitated by acids. Pectin forms a jelly when its aqueous solution is faintly acidified, while gum tragacanth merely swells up when treated with cold water without undergoing notable solution.

The investigations of O'Sullivan have shown that the gums are not carbohydrates of the formula $(C_6H_{10}O_5)_n$, as at one time supposed, but in reality glucosidic derivatives of certain organic acids and built up of the residues of sugar molecules united by ethereal oxygen to the organic acid. This acid is different in different gums and is to be regarded as the nucleus of the particular gum; thus gum arabic contains arabic acid, $C_{23}H_{38}O_{22}$; geddah gum gives geddic acid, an isomeride of arabic acid; gum tragacanth gives bassoric acid, $C_{14}H_{20}O_{13}$. The commonest sugars in gums are galactose and arabinose. According to Voley-Boucher (Bull. de Sciences Pharmacol., 1908, 15, 394), most gums contain a soluble enzyme capable of decomposing amygdalin. Soluble gums are more active than insoluble ones.

A natural gum is often a mixture of several gum compounds differing in the number of sugar residues in their molecules. A summary of the present position of the chemistry of the gums is given by H. H. Robinson (*Report Brit. Assoc.*, 1906 (York), 227).

In analysing gums it is necessary to identify the sugars and the gum acids. The principal constants of a gum acid are its ultimate composition, neutralising power for bases and optical rotation. As the acids do not crystallise, to prove their individuality it is necessary to precipitate them fractionally and to compare the constants of different fractions. The amount of mucic acid and of furfural yielded should be also determined. The following analyses of some tree gums are due to Huerre and Lemeland:

	Almond tree (hard gum)	Almond tree (elastic gum)	Apricot tree	Plum tree
Soluble in water, Calculated on dry gum,	21.06	8.9	91.17	79.16
Insoluble in water,	78.94	91.1	8.83	20.84
Loss at 100°,	15.76	25.0	16.14	15.48
Ash,	2.34	....	3.39	2.52
Galactans as galactose,	23.7	....	23.6	16.36
Pentosans as arabinose,	54.6	....	48.57	76.35
Total sugars,	85	91	78.7	94.8
Sugars identified,	Arabinose and galactose	Arabinose and galactose	Arabinose	Arabinose

Gum Arabic. Gum Acacia.—Gum arabic is the dried exudation from the bark of various species of *Acacia*. Strictly speaking, "gum arabic" is the generic name, "gum acacia" being properly limited to the superior qualities employed in medicine. These are largely obtained from the Soudan. The finest kind of gum arabic occurs in commerce in lumps of various sizes, colourless and full of minute cracks. Gum Senegal forms yellowish or reddish lumps, often of the size of a pigeon's egg, and not having the minute cracks of the better varieties. It is less readily soluble than true gum arabic, and its solution soon becomes very dark in colour.

Gum arabic consists essentially of calcium arabate or arabic acid (arabin), which may be obtained pure by dialysing a solution of the gum previously acidulated with hydrochloric acid. The colloid liquid thus obtained is lævorotatory, and is not precipitated by pure alcohol, but is thrown down if a trace of any acid or salt be present.

After being evaporated to dryness and heated to 100°, arabin does not redissolve, even in hot water, but swells up into a gelatinous mass, which gradually dissolves on treatment with alkalies, or alkaline earths, in presence of water, yielding a liquid indistinguishable from the aqueous solution of ordinary gum arabic.

Most varieties of gum arabic—including the Levantine, Sennaar, East Indian and Senegal—are lævorotatory, but Australian gum is often optically inactive, while Gedda gum is dextrorotatory. Chemically, these gums are analogous to the dextrorotatory varieties.

The inferior qualtities of gum contain a small percentage of a reducing sugar, which may be removed by treatment with alcohol.

The sp. gr. of air-dried gum arabic ranges from 1.35 to 1.49, but when completely dried at 100° it loses about 13% of water, and the sp. gr. increases considerably.

Gum arabic has a very faint odour and a mucilaginous insipid taste. It dissolves slowly in about twice its weight of water, forming a thick transparent mucilage of acid reaction. Gum is slightly soluble in dilute spirit, but quite insoluble in liquids containing more than 60% of alcohol, and is precipitated from its aqueous solution on addition of a large proportion of spirit.

The aqueous solution of gum arabic is not precipitated by neutral lead acetate, but with the basic acetate it forms a white jelly. Its solution is also precipitated by potassium or sodium silicate, borax, ammonium oxalate, mercuric chloride and ferric salts.

Suakim gum, which is quite brittle, is often not wholly soluble in water, but yields with it a pasty mass of rather strong acid reaction, depositing, when diluted with water, transparent globules, said to consist of metagummic acid which may be rendered soluble by adding a little potash or lime-water.

The proportions of mucic acid obtainable from the different varieties of gum by oxidation with nitric acid have been determined by Kiliani (*Ber.*, 1882, **15**, 34), who found amounts varying from 14.3%, from a sample of East Indian gum, to 38.3%, from an Australian sample.[1]

[1] The treatment of the gums with nitric acid was conducted in the following manner: 2 grm. of the powdered sample were digested at 60° with 5 c.c. of nitric acid of 1.2 sp. gr. until the whole became a solid mass saturated with the liquid. Another 5 c.c. of nitric acid were then added and the liquid filtered. The residue of mucic acid was washed thoroughly, dried at 100°, and weighed. The filtrate and washings were evaporated together and again treated with nitric acid, when a further quantity of mucic acid was obtained while a third treatment generally yielded only a trace in addition.

By adding a saturated solution of aluminum sulphate to one of gum arabic, the adhesive properties of the latter are said to be much increased, owing to the formation of aluminum arabate, while calcium sulphate is gradually deposited.

The presence of gum arabic in a solution presents the formation of a number of characteristic precipitates (Lefort and Thibault, *Pharm. Jour.*, [3] 1882, **13,** 301), a fact which is of importance in toxicological researches. Thus, in presence of gum arabic, dilute solutions of mercury, lead, copper, silver, iron, and arsenic, do not give precipitates with hydrogen sulphide or alkaline sulphides, though the liquids acquire a colour corresponding to the sulphide which would otherwise be precipitated. The precipitation of calcium phosphate and uranyl ferrocyanide is prevented in a similar manner, while in presence of gum arabic the alkaloids are not precipitated by sodium phosphomolybdate, potassium mercuric iodide or tannin.

Assay of Gum Arabic.—Gum arabic should not contain more than about 4% of *ash.* It should be soluble almost without residue in cold water. The solution should be free from *starch* and *dextrin,* which may be ascertained by the negative reaction with iodine solution; but should be rendered turbid by oxalic acid, which the solution of dextrin is not. The better kinds of gum arabic do not reduce Fehling's solution when heated to boiling with it, any red precipitate being due to the presence of a reducing sugar, small quantities of which exist naturally in certain inferior varieties of gum, though any considerable quantity has probably been introduced as an impurity in an admixture of *dextrin.*

According to Z. Roussin (*J. Pharm. Chim.*, [4] 1868, **7,** 251), *gum arabic* and *dextrin* may be distinguished and separated by means of ferric chloride, which precipitates the former only. The solution is concentrated to a syrup, mixed with ten times its volume of rectified spirit, and the resultant precipitate washed with rectified spirit and dried. 1 grm. of the dry residue is then dissolved in 10 c.c. of water, the solution mixed with 30 c.c. of proof spirit, 4 drops of ferric chloride solution (containing 26% of the anhydrous chloride) added, followed by a few decigrammes of powdered chalk; and after stirring briskly and leaving the liquid at rest for a few minutes it is filtered. The precipitate is washed with proof spirit, and the dextrin is precipitated from the filtrate by adding very strong alcohol. After 24 hours the spirituous liquid is decanted, the dextrin dissolved in a

small quantity of water, the resultant solution evaporated at 100°, and the residue weighed. The precipitate containing the gum must be dissolved in dilute hydrochloric acid, the arabin precipitated by adding absolute or very strong alcohol, and after washing with spirit is dissolved in water, the solution evaporated, and the residue weighed. The precipitation of gum arabic from a dilute alcoholic liquid by ferric chloride and chalk is so complete that nothing but calcium chloride can be found in the filtrate, while the precipitate similarly produced in a solution of dextrin is perfectly free from the latter body. By the formation of a cloud on adding ferric chloride alone, the presence of gum arabic is sufficiently demonstrated, while the clouding of the filtrate from the iron-chalk precipitate on addition of alcohol proves the presence of dextrin.

Another test by which gum arabic may be distinguished from dextrin is given on page 439. A large proportion of dextrin would be indicated by the dextrorotatory action of the solution, but the differences in the optical activities of natural gum arabic and commercial dextrin prevent the quantitative application of the test.

For the separation of gum arabic from *sugar*, Andouard dilutes 10 grm. of the syrup with 100 c.c. of spirit of 0.800 sp. gr., adds 20 drops of acetic acid, and stirs vigourously. After 3 hours the liquid is poured on a double filter, when the gum forms a cake which readily drains. This is dissolved in a little water, and the precipitation repeated, the precipitate washed with alcohol, dried at 100° and weighed. It is then exposed to the atmosphere for 24 hours, when it will have taken up its normal amount of moisture.

The inferior kinds of gum are largely employed as thickening agents in calico-printing. Good gum neither tarnishes nor alters delicate colours and does not weaken the mordants. The action of gums on delicate colours may be ascertained by printing a solution of the sample mixed with cochineal-pink or fuchsine upon pure wool; the fabric is then steamed and washed, when, if the gum be pure, there will be no trace of yellowness apparent. Too great an acidity of the gum gives it a solvent action on mordants, and hence renders it unsuitable for use.

The relative viscosity of samples of gum is an important character in judging of their quality. This may be tested by making solutions of 10 grm. of each sample in a little warm water, diluting the liquids to 100 c.c., and ascertaining the rate at which the solutions flow from

a glass tube drawn out to a fine orifice. A recently prepared solution of gum of the best quality should be used as a standard.

Gum tragacanth is the gummy exudation from certain species of *Astragalus*. It occurs in flattened, tear-like masses, strings or curved bands, which are usually marked with ridges or other indications of lamination.

According to Giraud, gum tragacanth usually contains about 60% of a pectinous body which yields pectic acid by boiling with water containing 1% of hydrochloric acid; from 8 to 10% of soluble gum, probably arabin; 5 to 6% of starch and cellulose; 3% of ash; 20% of water, and traces of nitrogenous bodies. The ash is chiefly calcium carbonate.

The characteristic pectinous constituent of gum tragacanth is known as tragacanthin, adracanthin, or bassorin, and is stated to have the composition $C_{12}H_{20}O_{10}$.

Tragacanth is usually white or yellowish (having sometimes been bleached by chlorine), but the inferior varieties have a brownish colour. It is hard, tough and difficult to powder. Tragacanth is odourless and tasteless and insoluble in alcohol or ether. With 50 parts of water it swells up and forms a thick, jelly-like mucilage, without actually dissolving. When diffused through a much larger quantity of water it forms a ropy liquid which may be passed through a filter, leaving an insoluble residue which is coloured blue by iodine from the presence of starch. Mucilage of tragacanth is coloured yellow by caustic soda, and a solution of the gum yields clear mixtures with borax, alkaline silicates, and ferric chloride, but is precipitated by alcohol. It becomes thick on adding neutral or basic lead acetate, and on heating the mixture a precipitate is formed.

Before being used for calico-printing, gum tragacanth is swelled by soaking in cold water for 24 hours, and afterwards boiled with water for 6 hours, when a thick homogeneous solution results, which, however, has but little cohesive power. The comparative viscosity of the liquid can be ascertained as in the case of gum arabïc (page 443).

To distinguish gum acacia and gum tragacanth advantage is taken of the fact that the latter contains no active oxidase such as is present in gum acacia. A cold aqueous solution, 1 in 30 of the gum, is treated with an equal volume of 1 % aqueous solution of guaiacol; 1 drop of hydrogen peroxide solution is then added and the mixture shaken. In presence of gum acacia the liquid rapidly acquired a brown tint,

whilst with pure gum tragacanth it remains colourless. (Payet, *Ann.. Chim. Anal.*, appl. 1905, **10**, 63.)

The gum of *Cochlospernum gossypium* is remarkable for its power of slowly giving off acetic acid when exposed to moist air or on hydrolysis (H. H. Robinson, *Trans. Chem. Soc.*, 1906, **89**, 1496). It yields further a gum acid (gonadic acid), xylose and galactose. This property is also possessed by the gum of *Sterculia urens*.

PROXIMATE ANALYSIS OF PLANTS.

The analysis of plants, as a rule, resolves itself into the qualitative detection and quantitative separation of some single substance or group of substances. So far as the carbohydrates are concerned the subject has been fully dealt with in the preceding pages and the alkaloids and similar groups of substances will be treated elsewhere. At the present time, biological methods are much used and it is also becoming customary to test plants for enzymes.

The following scheme, as practised in the laboratory of Prof. A. B. Prescott, is intended to facilitate the systematic analysis of vegetable substances. Substances having by its aid been proved to be present should be isolated by methods specially devised for each individual case. Owing to the very rapid changes which take place in plants after being gathered or on being macerated with water owing to the action of enzymes, it is often advisable to destroy the enzymes immediately; this is conveniently done by dropping the freshly cut plant piecemeal into boiling alcohol.

Moisture is estimated by methods given on page 64. The loss of weight may include a little volatile oil.

Total nitrogen is obtained by the Kjeldahl process. This is multiplied by 6.33 to convert it into proteins. It must not be assumed, however, that all the nitrogen present exists as proteins, the contrary being commonly the case. An outline of the method of estimating the nitrogen existing in various forms is given by Schulze and Barbieri (*Landw. Versuchs-Stat.*, 1881, **26**, 213), but if alkaloids are present they must be isolated by separate means.

Action of Solvents.—The substance is then submitted to a systematic treatment with solvents and reagents in the manner prescribed in the following tables:

A. SOLUTION IN BENZENE OR CHLOROFORM.—Evaporate carefully to dryness, and weigh the residue. Then treat with water; again evaporate to dryness at 100°, heat to 110°, and weigh again.

Volatilised.—Volatile oils, camphors (partially), volatile alkaloids. The last may be detected by the alkaline reaction of the aqueous liquid and their loss avoided by adding a drop of hydrochloric acid before evaporation.

Residue.—Treat with a moderate quantity of warm water, and when cold filter through fine paper by Bunsen pump.

Solution.—Divide into two equal portions, *a* and *b*:

a Evaporate to dryness, and weigh total extract. Ignite, and weigh ash.

b. Test portions for alkaloids and glucosides by special reagents; and for organic acids by solutions of barium, calcium, iron, lead and silver.

Residue.—Remove from the filter and vessels used by benzene or chloroform, and agitate solution with warm, very dilute hydrochloric acid, and separate by means of a tapped funnel.

Acid Solution.—Test for alkaloids and glucosides.

Benzene Solution.—Evaporate to dryness, and treat residue several times with spirit of 0.848 sp. gr. Filter through paper.

Solution may contain camphors resins, chlorophyl, certain fixed oils (*e. g.*, castor oil). Camphors are recognisable by the smell; chlorophyl by its absorption spectrum.

Residue consists of fixed oils, fats, wax, and, very rarely, resin.

Treat 5 grm. of the finely-divided substance with benzene wholly distilling below 86°, or, failing this, with chloroform. The treatment should be continued for six hours, and be conducted in a Soxhlet's extractor or other suitable apparatus for repercolation.

A. *Solution* may contain alkaloids, glucosides, free organic acids, chlorophyl, certain resins, fixed oils, fats, and waxes, camphors, volatile oils, but no mineral matter.

Residue.—Dry at 100°, weigh and treat with redistilled methylated spirit of 0.848 sp. gr. for twelve hours in a Soxhlet's tube.

B. *Solution* may contain mineral matters, tannin, organic acids, alkaloids, glucosides, certain extractive and colouring matters, resins, and sugars.

Residue.—Dry at 100°, weigh and treat with a known measure of cold water. Macerate, with frequent agitation, for eight or ten hours. Then filter through fine washed linen or paper if possible.

C. *Solution* may contain soluble proteins, gum; and, in the analysis of fruits and fleshy roots, pectin bodies, salts of organic acids, dextrinoid bodies, and colouring matters.

Residue.—Wash with alcohol, dry at 100°, and weigh. Then treat with 500 c.c. of water and 5 c.c. of concentrated sulphuric acid and heat till a drop of the liquid gives no colour with iodine.

D. *Solution* may contain dextrin and maltose from conversion of starch; also proteins and occasionally organic acids, either as salts or free.

Residue.—Wash thoroughly, dry at 110° and weigh. Boil for two hours with 500 c.c. of a 2 per cent. of sodium hydroxide. Filter through washed linen.

E. *Solution* may contain proteins matters, pectous matters, cutose, humus, and products of decomposition.

Residue.—Wash thoroughly in succession with hot water, alcohol and ether. Dry at 110° C. and weigh. Treat as directed on page 392.

F. *Solution.*—Lignin and colouring matter.

Residue.—Weigh as cellulose.

B. SOLUTION IN ALCOHOL OF 0.848 SP. GR.—Concentrate to a small bulk, and remove, dry and weigh any crystals or powder which may separate from the cooled liquid. Dilute the clear liquid to 200 c.c. by spirits of 0.848 specific gravity, and divide into several aliquot parts (20, 20, and 160 c.c.).

20 c.c.—Evaporate to dryness, and weigh total extract. Ignite and weigh again to determine ash and total organic extract.

20 c.c.—Evaporate nearly to dryness, add water, filter, and evaporate filtrate to dryness. Residue is soluble extract, and on ignition leaves the soluble ash.

160 c.c.—If much sugar or tannin is present (recognisable by the taste) employ process a: if but little of either of these be present use process b.

(a) Evaporate nearly to dryness, add water, filter and make up filtrate to 160 c.c.

(b) Evaporate carefully to dryness, pulverise and treat residue with several considerable portions of absolute alcohol (specific gravity 0.7938). Filter.

Residue.—Treat with water.

Residue may contain resins; colouring matters, proteins especially from seeds; alkaloids, and glucosides.

Solution.—Divide into eight portions of 20 c.c. each.
1. Precipitate tannin with ammoni-

Solution.—Evaporate nearly to dryness and add water.

Solution.—Add basic acetate of lead. Loss of weight on igniting precipitate represents tannin, organic acids and some extractives. Filtrate may contain alkaloids, glucosides, extractive and colouring matters.

2.

Solution.—Add basic lead acetate. Loss of weight on igniting precipitate represents coloured matters, extractives, organic acids, proteins (rarely). Filtrate, remove lead, and determine dextrose by Fehling's solution, and glucosides and sucrose by increased reduction after inversion.

Residue may contain:
1. Alkaloids glucosides (rarely), and extractives soluble in dilute hydrochloric acid.
2. Matters insoluble in dilute hydrochloric acid
3. Acid resins and colours soluble in dilute ammonia.
4. Neutral resins, colours and nitrogenous matters insoluble in dilute ammonia.

Residue.—Treat with dilute hydrochloric acid.
1. Dissolved; some alkaloids and glucosides.
2. Insoluble; some resins, and colouring and extractive matters. Dissolve in alcohol; evaporate to dryness, and weigh.

3

formed from glucosides and sucrose.
5 and 6. Treat with basic lead

use
ith
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ter

to

7 other portions.

C. SOLUTION IN COLD WATER.—Make up liquid to known volume and divide into aliquot portions.

1. *Total solid matter* obtained by evaporating and drying residue at 110°; *ash* by ignition.

2. Add solution of iodine. Blue indicates *soluble starch;* reddish-brown, *erythrodextrin.*

3. Add ammonium oxalate. A white precipitate indicates calcium, probably as calcium arabinate.

4. Evaporate a known volume, apply Keldahl method, and multiply nitrogen found by 6.33 to obtain proteins.

5. Add dilute hydrochloric acid. A gelatinous precipitate consists of *pectin* or *pectic acid;* if the liquid be filtered and treated with four times its measure of alcohol, a further precipitate may consist of *arabin* or *dextrin.*

D SOLUTION IN DILUTE ACID.—Boil with excess of barium carbonate, exactly neutralise last traces of acid by cautious addition of solution of barium hydroxide, filter, concentrate, and bring volume to exactly 50 c.c. Then ascertain sp. gr., and divide excess above 1000 by 8. The figure thus obtained is the weight of starch in the 5 grm. of substance taken. If the sp. gr. indicates but a small proportio nof starch, treat half the solution with 1 c.c. of concentrated sulphuric acid, and heat the liquid to 100° for 3 or 4 hours; then neutralise, and estimate glucose by Fehling's solution. The amount found, multiplied by 0.9. gives *starch.* Test portion of original neutralised solution by adding tannin. A white or buff precipitate indicates *proteins.*

E. SOLUTION IN DILUTE ALKALI.—Add slight excess of hydrochloric acid. A precipitate may contain *pectic acid* and other bodies, such as colouring matters. Further precipitation usually occurs on adding alcohol.

J. M. Albahary (*Compt. rend.*, 1908, **146**, 336) has recently given the following scheme for the complete analysis of vegetable substances. A portion is dried at 100° to obtain the quantity of volatile matter, and is then incinerated to give the amount of total ash. A second portion of the sample is extracted with alcohol; the alcoholic extract is distilled at a low temperature, and the distillate is collected in a receiver containing a known volume of standard sodium hydroxide solution and surrounded by a freezing mixture. On titrating back the excess of sodium hydroxide, the quantity of volatile acids is obtained, and this added to the weight of the residue remaining in the distillation flask gives the weight of the alcohol-soluble substances. The sum of the substances soluble and insoluble in alcohol subtracted from the weight of the original material gives the actual amount of the fat, colouring matters, cholesterol and lecithin. The portion of the substance insoluble in alcohol is next digested for 2 days in alcohol acidified with hydrochloric acid. The solution is then poured through a filter and the residue is washed with alcohol. The filtrate and washings are evaporated, the residue is weighed, extracted with ether to remove organic acids and then dissolved in water. Portions of the solution are used for the estimation of the reducing sugars, mineral acids, nitrogen, asparagine, sulphur and ash. In the portion insoluble in acidified alcohol are estimated the total protein, nuclein, albumin, starch, cellulose, etc.

The following books will be found to give useful special information. For general morphological and microscopic character of plants see Wiesner, *Die Rohstoffe des Pflanzenreiches;* for microscopic identification of technical fibres see Franz von Höhnel, *Die Mikroscopie der technisch-verwendeten Faserstoffe.*

CEREALS.

Owing to the defective methods employed, many of the older analyses of wheat and other grains are of doubtful value.

A. H. Church gives the following analyses by himself in illustration of the composition of representative specimens of the cereal grains and products therefrom:

	White English Wheat	Fine Wheat Flour	Wheat Bran	Scotch Oat-meal[1]	Pearl Barley[2]	Rye Flour	Cleaned Rice	Maize	Millet	Dari
Water	14.5	13.0	14.0	5.0	14.6	13.0	14.6	14.5	13.0	12.2
Proteins and other nitrogenous bodies	11.0	10.5	15.0	16.1	6.2	10.5	7.5	9.0	15.3	8.2
Starch with traces of Dextrin, etc	69.0	74.3	44.0	63.0	76.0	71.0	76.0	64.5	61.6	70.6
Fat	1.2	0.8	4.0	10.1	1.3	1.6	0.5	5.0	5.0	4.2
Cellulose and Lignose	2.6	0.7	17.0	3.7	0.8	2.3	0.9	5.0	3.5	3.1
Mineral matter	1.7	0.7	6.0	2.1	1.1	1.6	0.5	2.0	1.6	1.7
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

For convenience of comparison, the following analyses of other vegetable products are given. They are selected from among a large number published in Church's work on *Food*:

	Buck-wheat	Peas	Haricot beans	Lentils	Earth-nut shelled
Water	13.4	14.3	14.0	14.5	7.5
Proteins	15.2	22.4	23.0	24.0	24.5
Starch	63.6	51.3	52.3	49.0	11.7
Fat	3.4	2.5	2.3	2.6	50.0
Cellulose and lignose	2.1	6.5	5.5	6.9	4.5
Mineral matter	2.3	3.0	2.9	3.0	1.8
	100.0	100.0	100.0	100.0	100.0

	Potatoes	White turnips	Carrots	Beet-root Red	Yam
Water	75.0	92.8	89.0	82.0	78.6
Proteins	2.3	0.5	0.5	0.4	2.2
Sugar	....	....	4.5	10.0	
Starch	15.4	....	....	....	16.3
Dextrin, gum and pectose	2.0	4.0	0.5	3.4	
Fat	0.3	0.1	0.2	0.1	0.5
Cellulose and lignose	1.0	1.8	4.3	3.0	0.9
Mineral matter	1.0	0.8	1.0	0.9	1.5

More recent analyses have been published by the United States Department of Agriculture, from the bulletins of which the following figures are taken:

[1] One hundred pounds of oats yield about 60 of oatmeal and 26 of husks, the remainder being water and loss.
[2] The product called pearl barley constitutes only about one-third of the whole seed.

COMPOSITION OF CEREAL GRAINS.

	Moist-ure	Pro-teins (6.25N)	Ether ex-tract	Crude fibre	Ash	Carbohy-drates other than crude fibre
Typical unhulled barley.........	10.85	11.0	2.25	3.85	2.5	69.55
Typical American maize........	10.75	10.0	4.25	1.75	1.5	71.75
Typical wheat.................	10.6	12.25	1.75	2.4	1.75	71.25
Sweet corn, 19 samples (Richard-son).......................	8.44	11.48	8.57	2.82	1.97	66.72
Typical American buckwheat....	12.0	10.75	2.0	10.75	1.75	62.75
Typical unhulled oats..........	10.0	12.0	4.5	12.0	3.4	58.0
Typical rye...................	10.5	12.25	1.5	2.1	1.9	71.75
Typical rice, unhulled..........	10.5	7.5	1.6	9.0	4.0	67.4
Typical rice, hulled but unpolish-ed...................	12.0	8.0	2.0	1.0	1.0	76.0
Typical rice, polished..........	12.4	7.5	0.4	0.4	0.5	78.8

COMPOSITION OF FLOURS.

	Moisture		Ash		Proteins (6.25 N)		Fibre		Ether Extract		N-free Extract	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
Wheat........	15.0	9.0	0.8	0.3	15.0	8.0	1.0	0.1	2.0	0.5	90.0	82.0
Rye..........	14.0	12.0	1.5	0.5	11.0	6.0	0.6	0.4	1.0	0.9	92.0	88.0
Barley........	15.0	10.0	2.0	1.0	12.0	8.5	0.6	0.3	2.0	0.5	92.0	87.0
Buckwheat....	18.0	12.5	1.5	0.8	9.5	5.0	0.6	0.3	2.0	0.8	93.0	84.0
Rice..........	15.0	10.0	0.6	0.3	10.0	7.0	0.4	0.1	0.6	0.3	90.0	85.0
Oat (meal)....	10.0	6.0	2.4	2.0	18.0	14.0	1.4	0.7	9.5	6.5	76.0	72.0
Maize (meal)..	18.0	8.0	4.5	1.0	11.5	8.0	3.5	0.7	6.0	2.5	80.0	63.0
Graham........	15.0	11.0	2.2	1.8	15.0	10.0	2.4	2.0	2.2	1.9	72.0	70.0

Proteins of Cereals.—Although cereals are mainly composed of starch, it is the protein constituents which often impart to them their characteristic properties and which therefore are investigated in an analysis. Indeed, in very many of the published analyses, the starch is determined by difference. Although different proteins occur in different cereals, many of the proteins contain about 15.8% of nitrogen and it has become usual to deduce the proportions of proteins present by

multiplying the percentage of nitrogen by 6.33 $\left(=\dfrac{100}{15.8} \right)$. This pro-

cedure ignores the fact that the whole of the nitrogen of plants does not exist in the form of proteins and may be very misleading if the analysis is used to judge of the suitability of a cereal for bread mak-

ing or its food value. For wheat proteins the factor 5.7 is considered more accurate.

This subject will be fully dealt with in Volume VIII.

WHEAT.

Two varieties of wheat—*Triticum vulgare*—are cultivated, distinguished as spring and winter wheat. Individual species and samples differ rather widely in composition. König's table for the mean composition of wheat from 250 analyses is quoted here.

Water,	13.56%
Proteins,	12.42%
Fat,	1.70%
Starch,	64.07%
Sugar,	1.44%
Gums and dextrin,	2.38%
Fibre,	2.62%
Ash,	1.79%

Ground wheat free from bran is termed flour. The commercial value of wheaten flour depends on the colour and texture and upon the quality and to some extent the quantity of the contained gluten. In practice the somewhat elastic term "strength" is employed, which is defined as the power of flour to give a shapely well-risen loaf (Humphries and Biffen) or as the power of flour to absorb water when made into dough (Jago). Bakers prefer a flour with a high percentage of tenacious gluten. The causes of strength are as yet imperfectly understood.

FLOUR.

Good wheat flour should be an almost perfectly white impalpable powder with only the very faintest yellow tinge. When pressed smooth by means of a polished surface, no traces of bran should be visible; it should have a sweet odour and flavour and be free from acidity. When comparing flours the following tests may be made:

Colour.—Small wedge-shaped heaps with a smooth surface are arranged side by side on a sheet of glass and the colours compared when dry and after carefully immersing the plate in water, when, as a rule, the colours are much more marked.

Doughing Test.—A known weight of the sample is made into a dough with 1/2 to 2/3 its weight of water and the colour and "feel," that is firmness, elasticity and compactness, of the doughs are compared.

Gluten Test.—To obtain results comparable at different dates much care must be paid to detail in carrying out this test. About 30 grm. of flour are made to a stiff dough with 12–15 c.c. water and allowed to stand for 1 hour. The mass is then carefully kneaded in a stream of running water until all the starch has been removed. This kneading is conveniently carried out between the fingers, the flour being held over a sheet of fine muslin which allows the starch to pass but retains any particles of gluten which may fall on it. Other operators advise kneading the flour wrapped in a piece of linen. The ball of fresh gluten thus obtained should be tough and elastic, capable of being pulled out in threads and but little coloured. Flour from English wheats gives a very soft and sticky gluten showing very little elasticity, whereas that from Canadian spring wheats is very tough and elastic. After washing, the gluten is left for an hour under water, the excess of moisture is then removed between the hands as far as possible and the wet gluten weighed. It is then dried for 40 hours at 98° C. or for a shorter period at a higher temperature and the weight of dry gluten determined.

This crude gluten consists in reality of true gluten together with small percentages of non-gluten proteins, mineral matter, fat, a little starch, fibre, etc.

ANALYSIS OF FLOUR.

1. **Moisture** is determined by heating at 100° until no further loss in weight occurs.

2. **Fat** is determined by ether extraction of the dry flour. A high value for the ether extract of a patent flour (above 1.5%) indicates incomplete removal of germ particles.

3. **Gluten** may be estimated approximately by washing as described above or by a nitrogen determination on the original flour. The percentage of nitrogen found is multiplied by the factor 5.7. (This is preferable to 6.33 in the case of wheat.)

4. **Ash** is best determined in a muffle or the flour may be mixed with ammonium nitrate and burnt. If above 1%, mineral adulteration is probably present.

5. **Starch** may be estimated by one of the methods already described, see page 420.

6. **Gliadin** may be determined by extracting with 70% (by volume) alcohol for 2 hours and determining nitrogen in the filtrate. This is multiplied by the same factor as the gluten nitrogen.

7. **Cold water extract,** which consists of sugars, soluble proteins and potassium phosphate, etc., is obtained by digesting flour with a large volume of water, filtering and evaporating an aliquot portion in a weighed dish. The residue is ignited to give the soluble ash. The result depends largely on the temperature and time of extraction, as under these conditions the diastatic capacity of a flour causes a rapid increase in the amount of soluble sugar.

8. **Acidity** is best determined by direct titration with decinormal alkali, using phenolphthalein as indicator. Normal wheat has an acidity 0.16 to 0.25%, calculated as lactic acid. 20 grm. of flour are shaken with 200 c.c. of water for 2 hours filtered and 50 c.c. of the filtrate titrated. The test is most useful for detecting unsound wheat and flour.

9. **Diastatic Power.**—0.4 gram. flour and 200 c.c. of a 2% solution of soluble starch are maintained for an houi at 15.5°. Action is stopped by a drop of ammonia and portions of 2 c.c. are heated in test-tubes in boiling water for 5 minutes with different quantities of Fehling's solution to determine the amount which is just reduced by the sugar formed. The diastatic power is 100 when 2 c.c. reduce 4 c.c. of Fehling's solution. It ranges in commercial flours from 25 to 60.

Gluten consists of two proteins: gliadin, remarkable for its solubility in dilute alcohol, and glutenin, which is soluble in very dilute alkali. Gliadin in the hydrated condition is a soft, sticky substance which can readily be drawn into threads, when dehydrated it forms a white friable mass. It may be prepared by extraction of flour or gluten with 70% (by volume) alcohol and precipitation from this solution by sodium chloride. It is readily soluble in pure water. Gliadin forms a sticky mixture with water, and the salts naturally present in the flour prevent its solution. The glutenin imparts solidity to the gluten, evidently forming a nucleus to which the gliadin adheres.

The ratio of gliadin to total gluten varies in different flours and authorities are not agreed as to the correlation of this ratio with strength. A flour with a high gliadin ratio is considered the best: Fleurent suggests 75%, Snyder 60%, but these values are not accepted by authorities in England.

Mineral Constituents of Wheat and Flour.—Whole wheat contains a much higher percentage of ash than flour. The ash of wheat ranges from 1.4 to 1.9%, and that of good flour seldom exceeds 0.7% or 0.85% in the case of seconds flour. It may be safely assumed that any flour, free from a notable proportion of bran, which yields a higher ash than 1% is adulterated.

Blythe quotes the following table as the mean composition of the ash of entire wheat:

	Winter wheat.	Summer wheat.
Potassium oxide,	31.16	29.99
Sodium oxide,	2.25	1.93
Calcium oxide,	3.34	2.93
Magnesium oxide,	11.97	12.09
Ferric oxide,	1.31	.51
Phosphoric oxide,	46.98	48.63
Sulphur trioxide,	.37	1.52
Silica,	2.11	1.64
Chlorine,	.22	.48

According to Snyder, the ash of wheat flour bears a proportion to the grade—the lower the grade the higher the percentage of ash. First patents have less than 0.4%, second patents less than 0.5% and a straight grade flour should not have more than 0.55% of ash. Mixing or misbranding of flours can be more accurately determined in this way than by any other means. The figures quoted refer to hard Canadian spring wheat.

The United States standard of purity is not more than 13.5% of moisture, 1% of ash and 0.5% of crude fibre and not less than 1.25% of nitrogen.

Adulterations of Flour.—Flour may be adulterated with mineral matters to increase its weight, with alum or copper sulphate to improve its appearance or with cheaper flours or starches. In exceptional cases it may contain weeds or have been damaged by mould and contain ergot.

The amount of *ash* affords a convenient and accurate means of detecting mineral adulterants with the exception of alum which is usually employed in too small a quantity sensibly to affect the percentage obtained. The mineral adulterants may be separated from a flour by shaking it with chloroform in a separating funnel and leaving it till the flour has risen to the surface. Any mineral adulterant sinks in the

chloroform and may be removed and examined. It is further purified by a second treatment with chloroform; the residue is obtained on a watch-glass, the chloroform removed by evaporation, and the solid weighed.

Alum or other crystalline matter is detected by microscopic examination; the residue is dissolved in a little cold water and filtered and the insoluble matter ignited and weighed. It should not exceed 0.1%, if the flour is free from insoluble mineral adulterant. The solution is evaporated to dryness and the crystals of alum observed; they may be tested for aluminium, sulphates, potassium and ammonium or the alum may be recognised by its astringent taste and reaction with logwood.

Logwood Test for Alum.—1 grm. of freshly cut fine logwood chips is digested for 10 hours in 30 c.c. alcohol; 10 c.c. of this extract are mixed with 150 c.c. water and 10 c.c. of a saturated solution of ammonium carbonate; 50 grm. of flour are made into a thin paste with water, a few drops of *fresh*[1] alkaline logwood solution added and the mixture put aside for some hours. Alum produces a lavender-blue lake, pure flour a pinkish colour which fades to a dirty brown. The test is sensitive to $\frac{1}{10000}$ part of alum. The blue colour should remain when the sample is heated for an hour or two in the water-oven.

Wynter Blythe uses small strips of gelatin on which to concentrate the alum. A strip is soaked for 12 hours in the cold extract of the suspected flour and then taken out and steeped in the ammoniacal logwood, when, if alum is present, it acquires a very marked blue colour. The strips may be washed, dissolved in hot water and the absorption spectrum of the solution observed.

Alum acts in increasing the whiteness and improving the apparent quality of inferior flour. Its presence in flour or bread is always to be regarded as a sign of adulteration. (See under Bread.)

Copper Sulphate can be detected, even when present in but very minute proportion, by soaking the bread in a solution of potassium ferrocyanide acidulated with acetic acid, when a purplish or red-

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[1] In employing the logwood test for alum, it is very important that the tincture of logwood should be freshly prepared, and that the test should be made immediately after mixing the logwood tincture with the solution of ammonium carbonate. Inattention to these essential points has caused the failure to obtain the blue with specimens undoubtedly containing alum. The subsequent drying also should never be neglected. With proper care, the test is exceedingly delicate, 0.02% of alum causing a distinct shade of blue, while with three or four times this proportion the reaction is wholly beyond question.

On the other hand, a blue colouration of bread and flour by an ammoniacal solution of logwood does not infallibly prove the presence of a soluble aluminium compound, as several other mineral additions produce a somewhat similar reaction.

dish-brown colouration will be produced if copper is present. The amount of copper may be estimated by moistening 100 grm. of the bread with sulphuric acid, igniting and estimating the metal in the ash.

Very minute proportions of copper have been stated to exist normally in wheat-ash.

Plaster of Paris is readily separated from flour by treatment with chloroform.

Ergot in Flour.—The use of mouldy wheat in manufacturing flour may often be detected by moistening the sample and keeping it in a tightly closed vessel for some hours at about 30°, when any mouldy taint can readily be detected.

To test for ergot or any fungus, Vogel advises microscopic examination of the flour after staining with aniline violet. Starch granules that have been injured by the fungus acquire an intense violet tint, sound granules remain relatively colourless. Grüber heats a little of the moistened flour on a microscopic slide to the b. p. and examines with a power of 120 diameters when cold. Ergot may be identified by its high refracting power, furrows and colour—deep violet on the edge, greenish-yellow inside.

Chemical Tests.—20 grm. of flour are exhausted with boiling alcohol in a Soxhlet or other suitable apparatus until the last extract is colourless, and 1 c.c. of cold dilute sulphuric acid added. In the presence of ergot the solution will be red and when examined by the spectroscope in dilute solution will show two absorption bands: one in the green near E, and a broader and stronger band in the blue between F and G. On diluting the alcohol with a large volume of water the colour may be extracted from separate portions by ether, amyl alcohol, chloroform and benzene.

10 grm. of flour are digested for half an hour with 20 c.c. of ether and 10 drops of dilute sulphuric acid (1:5). The liquid is filtered and the residue washed with ether until 15 c.c. of filtrate are obtained. This is shaken with sodium hydrogen carbonate which takes on a deep violet colour if ergot be present, whereas the chlorophyl remains in the ether.

BREAD.

Bread is the flour of wheat made into a paste by kneading with water and permeated with carbon dioxide, produced as a rule by fermentation with yeast, but also by other methods. On baking the

gluten swells and retards the escape of the gas which expands little
cells and gives to bread the familiar light, spongy appearance. The
outside of the loaf becomes as hot as 210°, and is to some extent cara-
melised; the inside crumb is seldom raised much above 100°. The
amount of water in a loaf varies from 30 to 40% on the average, as
shown by the following analyses of wheaten bread collected by König
and taken from Wynter Blythe.

	Minimum	Maximum	Mean for fine bread	Mean for coarse bread
Water............................	26.39	47.90	38.51	41.02
Nitrogenous substances..........	4.81	8.69	6.82	6.23
Fat.............................	0.10	1.00	0.77	0.22
Sugar...........................	0.82	4.47	2.37	2.13
Carbohydrates...................	38.93	62.98	49.97	48.69
Fibre...........................	0.33	0.90	0.38	0.62
Ash.............................	0.84	1.40	1.18	1.09

The moisture depends, among other conditions, on the quality and
quantity of the gluten and the size and shape of the loaf.

The ash of a wheaten flour loaf seldom exceeds 1.5%; beyond 2%
would indicate mineral adulteration. A small quantity of common
salt is added to bread during manufacture.

Bread is relatively seldom adulterated. To test for alum, bread is
moistened with water and then with alkaline logwood solution (p. 457);
if alum be present the bread becomes lavender-blue in an hour or two.
The crust and crumb should be analysed separately, as an alumned
flour has been known to be used for dusting and facing the sponge
before baking it. To search for alum in crust it must be burnt to ash.

Blythe has found that a certain proportion of alum may always
be washed out of the bread as alum. 100 grm. of bread are soaked in
water for about 24 hours, the liquid strained through muslin and con-
centrated in a platinum dish. A strip of gelatin is steeped in a portion
on overnight and the logwood test applied when a blue is obtained,
if alum be present, to the extent of 0.03%. Allen suggested to
to dissolve the starch by malt extract, remove soluble carbohy-
drates by yeast, acidify with nitric acid, filter, evaporate, ignite
the residue and precipitate as phosphate in the usual way.

Alum is estimated by the Dupré-Wanklyn method as follows: 100
grm. of bread are ashed in a platinum dish, boiled with 3 c.c. of

strong hydrochloric acid and 30 c.c. of water; filtered and the precipitate (chiefly silica and unburnt carbon) washed, dried, burnt and weighed. 5 c.c. of ammonia are added and the calcium, magnesium, iron and aluminum phosphates precipitated. The liquid is *strongly acidified* with acetic acid, boiled and filtered and the insoluble phosphates remaining are washed and dried and weighed. The precipitate is redissolved and the iron estimated colourimetrically with ammonium sulphide, calculated as phosphate and subtracted from the total to give the weight of the aluminum phosphate.

An alternative method consists in burning the ash, boiling with hydrochloric acid and filtering as above. The filtered solution is again boiled and poured hot into·a very strong solution of sodium hydroxide, the mixture being again boiled. and filtered while hot. A little disodium hydrogen phosphate is added to the filtrate which is then slightly acidified with hydrochloric acid and finally made just alkaline by ammonia. The precipitate of aluminum is filtered, washed, ignited and weighed.

Flour normally contains a small proportion of aluminum in the form of silicate. It is customary, therefore, to determine the silica, subtract it from the amount of alum calculated from the aluminum phosphate found and multiply the remainder by 3.87 or 3.71 to give approximately the potassium or ammonium alum, respectively. (For further information on this question see Blythe's "Foods.")

The use of porcelain vessels is to be avoided throughout the process and care taken that the alkaline liquids are not heated in glass and that the sodium hydroxide used is scrupulously free from alumina.

The presence of plaster of Paris in bread is recognised by the high total ash and the high proportion of calcium contained in it. The sulphates of the ash do not afford a means of accurately determining the amount of plaster present, as proteins furnish a notable quantity of sulphates on igniting the cereals. On the other hand, only traces of sulphates exist ready formed in the cereals, and hence the estimation of them in the unignited bread affords a means of measuring the plaster present. This method, though theoretically perfect, presents some difficulties in practice, owing to the difficulty of obtaining a solution of the sulphates fit for precipitation with barium chloride. The best way is to soak 12.20 grm. of the bread for some days in 1200 c.c. of cold distilled water till mould commences to form on the surface of the liquid. The solution is strained through coarse muslin, and the

filtrate treated with 20 c.c. of phenol distilled over a small quantity of lime. The whole is then raised to the b. p. and filtered through paper. 1000 c.c. of the filtrate are then slightly acidulated with hydrochloric acid, and precipitated in the cold by barium chloride. 237 parts of barium sulphate represent 136 of plaster of Paris.

Detection of Bleaching Agents in Flour.—During the last few years there has been a widespread adoption of bleaching processes in the preparation of flour. Inasmuch as most bakers are accustomed to look upon the colour as the most reliable criterion of flour, the practice makes an inferior flour resemble a superior one. Many of the processes (those of Alsop and Andrews) make use of small quantities of nitrogen oxides in air to bleach the flour with or without ozone. Others involve the use of chlorine or bromine. In all cases of bleaching the agents probably form additive products with one or more of the constituents of the flour. The most delicate test for nitrites is the Griess-Ilosvay method (see page 241).

The following scheme will be found useful in testing flours for bleaching: 20 to 30 grm. of flour are extracted with an equal number of c.c. of benzene and the solution filtered into a porcelain dish. If colourless or nearly so, bleaching may be suspected. The solution is evaporated to dryness and the colour of the oily residue observed. An orange-red often indicates nitric oxide, whilst chlorine or bromine give a faint yellow or nearly white residue. An ignited bead of copper oxide is moistened in the oil residue and held in the bunsen flame when a bright green tinge confirms chlorine or bromine. 20 c.c. of water are added to the exhausted flour residue and 1 c.c. of the Griess-Ilosvay reagent added, when a very characteristic pink results if nitrous bleaching agents have been employed. The occasional use of nitrosyl chloride as a bleaching agent makes both tests desirable on the same sample.

MIXED FLOURS.

The addition of other flours to wheaten flour is somewhat uncommon, but may occur. The detection of such addition is a matter of considerable difficulty and requires patient examination under the microscope. Recent investigations, particularly those of Bigelow and Sweetser, Kraemer and Vogel have established certain data on which tests may be based.

Vogel advises extraction with 70% alcohol containing 5% hydro-

chloric acid. Pure wheat or rye flour give a colourless extract, with barley or oats a pale yellow extract, with pea flour an orange-yellow extract is obtained, mildewed wheat gives a purple-red and ergotised wheat a blood-red colouration.

Much may be ascertained from the gluten which is dark and viscous when rye flour is present, dark, non-viscous and dirty reddish-brown with barley, dark yellow with oats, yellowish and non-elastic with maize and varies from a greyish-red to green in the case of leguminous flours, such as bean or pea.

Leguminous starches give more ash than wheat flours; this ash is deliquescent, high in chlorides and turns turmeric paper brown. To detect legumin, the gluten is washed from a sample of the flour in the usual manner and the filtrate made alkaline with ammonia. It is allowed to stand overnight, the clear liquid decanted and the legumin precipitated by a dilute mineral acid. It may be collected, dried and weighed. According to Lemenant des Chenais, 0.9 grm. of legumin may be taken as indicating the mixture of 5% of leguminous flour.

A method of detecting potato flour in wheat is based on the resistance to destruction of the outer membrane shown by wheat flours. The sample is rubbed in a mortar with water to a stiff paste, which is then diluted and filtered. The filtrate tested with a drop of dilute iodine solution gives a deep blue with potato starch and a yellow or orange with pure wheat flour.

Maize.—Kraemer states that 5% of maize in wheat flour may be detected by mixing 1 grm. of the sample with 15 c.c. of glycerol and heating to boiling for a few minutes. Maize is indicated by the well-known odour of pop-corn.

A small quantity of the flour is treated with 10 c.c. of 1.8% potassium hydroxide for 2 minutes in a test-tube and then nearly neutralised with hydrochloric acid; wheat starch is gelatinised, maize remains intact.

Sawdust.—Le Roy (*Ann. Chim. anal.*, 1899, 4, 212) suggests the following test: 1 grm. phloroglucinol is dissolved in 15 c.c. 90 to 95% alcohol and 10 c.c. of syrupy phosphoric acid, 1 to 2 c.c. of this reagent are rubbed with a little of the sample in a porcelain dish, when sawdust assumes at first a rose and gradually a carmine tint. Pagamini (*Chem. Centr.*, 1905, 1, 695–696) moistens the flour spread in a thin layer first with a 0.2% aqueous solution of paraphenylenedia-

mine and then with acetic acid. The sawdust fragments are at once coloured orange-yellow.

To detect rice flour, Gastine (*Comptes. rend.*, 1906, **142,** 1207) advises staining with aniline blue or green, which shows up the hilum of the minute rice-starch granule as a reddish coloured point.

Other Cereals. (For analyses see page 452.)

Maize—Zea Mais.—Maize or Indian corn, though coming originally from America, has been largely cultivated in other countries. It is extensively used in the United States and its use is on the increase in Europe where it has been somewhat difficult to overcome the prejudice against it as it was first introduced as a food for lower animals. Corn starch is frequently met with in foods for invalids and infants. Maize is especially rich in fats, containing 5.2% according to the United States Department of Agriculture, and, roughly, twice as much oil as in wheat.

Oats—Species of Avena.—Oats are grown in northern regions throughout the world. They contain about 6% of fat and a high percentage of mineral matter. Oatmeal preparations are very largely used as breakfast foods and accordingly are found adulterated with other cereals, particularly barley. The admixture is only to be detected by microscopical investigation.

Barley—Species of Hordeum.—Barley is chiefly grown for the purpose of making malt. Both barley meal and "pearl barley," *i. e.*, the grain deprived of its outer coating and rounded by attrition, are used as foods, and barley meal is frequently used as an adulterant in other foods.

Rye—Secale cereale.—Rye bread has now almost fallen out of use in England, but it is the staple bread of the northern European nations. The fat is small in quantity and largely olein. The grain is particularly liable to become affected by ergot.

Rice—Oryza sativa.—Rice is the main food of a third of the human race. The term is applied to the seed separated from the hulls. It contains about 7% of proteins and 1% of fat and is readily digestible when cooked.

Bananas.—These combine the sweet qualities of a fruit with the nourishing properties of a vegetable, they are rich in sugar and starch and contain a fair quantity of proteins. A banana flour is made by drying the ripe fruit; this contains 4% proteins, 0.5% fat, 80% carbohydrates, 2.5% ash.

Of late a number of wheat and oat products have been placed on the market, particularly in the United States, as breakfast foods. These are either uncooked, partially cooked by steaming and drying or cooked and malted. The following analyses of some of these were made by Harcourt (*J. Soc. Chem. Ind.*, 1907, **26**, 240–243):

PERCENTAGE COMPOSITION OF SOME BREAKFAST FOODS.

	No. of samples analysed	Water	Crude protein	Crude fat	Carbo-hydrates	Crude fibre	Ash	Energy per grm. calories
Granulated oats.....	12	7.75	12.29	6.65	71.71	(1.59)	1.60	4.283
Rolled oats.........	19	8.55	11.83	6.61	71.35	(1.25)	1.66	4.238
Wheat farina........	8	10.63	9.70	1.05	78.23	(0.62)	0.57	3.876
Wheat germ	1	8.39	10.97	2.79	76.77	(1.15)	1.08	4.034
Rolled wheat........	2	10.41	8.77	1.90	77.22	(2.05)	1.70	3.860
Flaked barley.......	4	10.59	9.71	1.43	76.81	(2.07)	1.47	3.854
Cornmeal...........	2	9.76	6.99	1.26	81.49	(0.52)	0.50	3.870
Orange meat	3	8.66	9.70	1.31	78.43	(1.95)	1.90	3.909
Force	3	9.06	10.14	1.51	76.88	(1.85)	2.41	3.886
Norka..............	3	7.38	14.33	5.55	69.91	(1.84)	2.83	4.229
Malta Vita..........	3	8.23	9.88	1.39	78.27	....	2.23	3.915
Grape Nuts	3	7.08	11.49	0.94	78.78	....	1.71	3.995
Canada Flakes......	2	8.97	10.84	1.18	76.22	....	2.79	3.874
Shredded Wheat.....	2	9.41	11.53	0.85	76.51	....	1.70	3.916
Rice Flakes.........	1	12.29	7.24	0.08	80.04	(0.55)	0.35	3.716

PAPER AND PAPER-MAKING MATERIALS.

By R. W. SINDALL, F. C. S.

During the last few years the systematic application of chemical and microscopical analysis in the paper trade has become a matter of considerable importance. Prior to 1860 the manufacture of paper had proceeded along comparatively simple lines, the only raw material used in any quantity being rags, which required but little chemical treatment for conversion into paper. The high percentage of cellulose in cotton or linen rendered any drastic treatment unnecessary.

The wider application of chemical analysis in the paper trade can be traced to a number of causes, amongst which may be mentioned the introduction of new paper-making materials, such as esparto, straw and wood, requiring more severe treatment than the simple methods in vogue for the purification of rags. The increasing use of mineral substances, such as china clay and pearl hardening, and the question of adulteration by means of inferior fibres, together with a much greater variety in the classes of papers and allied products manufactured, widens the field of technical analysis. The lack of standards of quality and the importance of a study of the nature of chemical residues and fibrous ingredients as influencing the durability of the paper are now matters of common knowledge.

The methods of analysis peculiar to paper-making may be classified most conveniently under the heads, (1) the manufacturing process; (2) the finished paper.

The process of the manufacture of paper requires:

1. Some analytical methods which are of common application to all manufacturing industries.

2. Certain special methods of analysis peculiar to the paper trade.

The former need only be mentioned under suitable headings and the methods of analysis must be sought for in the proper text-books. The special methods required for paper analysis will be described at greater length.

In common with other industries, the efficient management of the paper-mill requires a systematic analysis of fuel, and other measurements usually applied in the production of steam for heating purposes and motive power.

The water supply is a question of paramount importance not only in regard to its suitability for steam boiler purposes, but more particularly in regard to its cleanliness and purity of colour for the manufacture of paper. The quantity of water required per ton of paper ranges from 5000 gallons in the case of cheap newspapers to 70,000 gallons per ton in the case of high-class rag papers. This difference, however, merely concerns the paper-mill, because the wood pulp utilised in the manufacture of newspaper has already been thoroughly washed before reaching the paper-mill.

The chemicals employed for the conversion of esparto, straw and other fibres into paper pulp are lime, crude sodium carbonate and crude sodium hydroxide. The sodium hydroxide is recovered by evaporation in a vacuum multiple effect apparatus and subsequent incineration of the concentrated mass, resulting in the formation of a crude carbonate. These materials are examined by the ordinary analytical methods.

The boiled pulp is treated with bleaching powder or with chlorine produced by an electrolytic process. The traces of bleach are occasionally removed by the use of an "antichlor," such as sodium hypochlorite, sulphurous acid and calcium hydrogen sulphites (bisulphites). The materials used for sizing the paper are glue, gelatin, casein, starch, rosin, rosin size and sundry mineral salts, to all of which the usual methods of analysis are applicable. Among the mineral substances used as ingredients of paper are calcium sulphate in its commercial forms, china clay, barytes, satin white (a mixture of calcium sulphate and precipitated alumina), asbestine, and French chalk.

Many of the common pigments, such as ultramarine, Prussian blue, smalts, ochre, as well as organic colouring matters are used in the manufacture of paper and these may be examined by ordinary methods.

THE TESTING OF PAPER.

The complete examination of a sheet of paper requires the measurement of its physical properties, the determination of the fibrous constituents and of other ingredients.

Physical Properties.

Weight.—The "substance" of a paper is expressed in terms of the weight of a ream of sheets of given area. A ream may contain 480, 500 or 516 sheets, according to the class of paper, high-class papers being reckoned as containing 480 sheets while cheaper papers are calculated for 516 sheets per ream. Many papers are cut to standard sizes having certain technical names, of which the following may be quoted as examples, the dimensions being in inches:

Double Crown,	30	x	20
Demy,	22.5	x	17.5
Foolscap,	17	x	13.5
Imperial,	30	x	22
Post,	21	x	16.5
Royal,	24	x	19

The description of a high-class paper as 28 pounds Double Crown means that a ream of 480 sheets, each measuring 20 x 30 ins., would weigh 28 pounds.

The weight of a paper can be measured by means of Leunig's paper scales, a full-size sheet being placed in the pan of the scales and the weight of the ream obtained by a direct reading on the scale.

The weight can also be ascertained by weighing on a delicate balance a small piece of known area and calculating the weight of a ream by simple proportion. The simpler expression of the weight of paper in terms of grms. per square meter or ounces per 1000 square ins. has not come into general use.
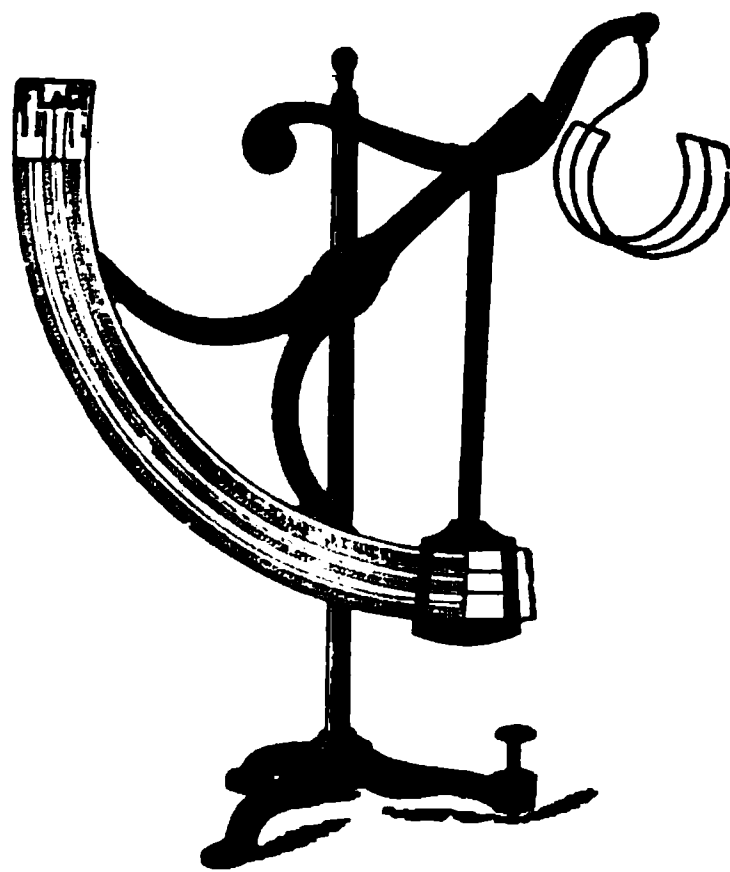
FIG. 72.—Leunig's paper scales

Thickness.—This is measured by means of a micrometer, special forms of which have been made for use in the paper trade. The thickness is expressed in inches or millimeters and sometimes in terms of the thickness of a ream.

Strength.—The strength of a paper may be measured in terms of

tensile strain—that is, the weight necessary to fracture a strip of given width—or in terms of *bursting strain*—that is, the number of pounds per square inch required to burst a sheet of paper rigidly fixed between suitable clamps.

Tensile Strain.—There are several machines for determining the breaking weight of a strip of paper, in all of which the strip, cut to

Fig. 73 —Leunig's paper testing machine

any convenient length and width, is fixed between clamps in a horizontal or vertical position, the tension being applied either by means of a weight or a spring. The machines are constructed to record the breaking weight at the moment of fracture, and also the amount of elongation produced in the paper while the tension is applied. The Schopper testing machine, in which a strip 25 mm. wide

and 180 mm. long is suspended vertically between the clamps and in which the tension is applied by means of a weight, is regarded as the standard instrument. With the Marshall paper testing machine, which is much used in England, the width of the strip may be from 0.25 in. to 2 in., and the length from 2 to 12 in., the paper being fixed in a horizontal position and the tension obtained by means of a spring.

Machine and Cross Directions.—In ordinary machine-made papers the strength of the paper will differ according to the position of the strip. The paper has a maximum strength in what is known as the "machine direction" and a minimum strength in what is known as the "cross direction." These terms are derived from the conditions of manufacture. The paper is formed by the felting together of minute fibres of pulp on the wire cloth of the machine travelling rapidly forward in a horizontal position. A strip cut from the sheet of paper along a

FIG. 74.—Marshall's paper testing machine.

line parallel with the direction in which the wet sheet of paper has been travelling on the machine is said to be cut from the "machine" direction. In the same way a strip cut at right angles to this is known as the "cross" direction.

The simplest method of determining exactly the machine direction of a sheet of paper is to cut a circular piece from the sheet, dampen one side by momentary contact with water, and then place the sample on the back of the hand. The damp paper will immediately curl up into a cylindrical form, the axis of the cylinder being parallel with the machine direction of the paper.

It is usual to make 5 or 10 tests for strength in the machine direction and a similar number in the cross direction, the mean of the figures being taken as the strength of the paper. It is obviously important to make a record of the strength in both directions.

Breaking Length.—In the absence of any definite standards for the length and width of the strip it is convenient to express the strength

of a paper in terms of its breaking length, *i. e.*, the length of strip which, if suspended, would break of its own weight. This factor is calculated by the formula:

$$\frac{w}{l} = \frac{W}{L}$$

in which   w = weight of test strip.

l = length of test strip.

W = breaking weight by experiment.

L = breaking length of paper.

Elasticity.—The elongation of a strip of paper produced by submitting the paper to tension is an important indication of its wearing qualities. The "stretch," as it is called, is measured simultaneously with the breaking weight, and the result is expressed in terms of the percentage of elongation, *i. e.*, the stretch per 100 units of length.

The word *stretch*, is frequently applied to the expansion of a paper, such as a lithographic printing paper, when wetted for printing. The term *expansion* would be preferable in the latter case and the two terms must not be confused.
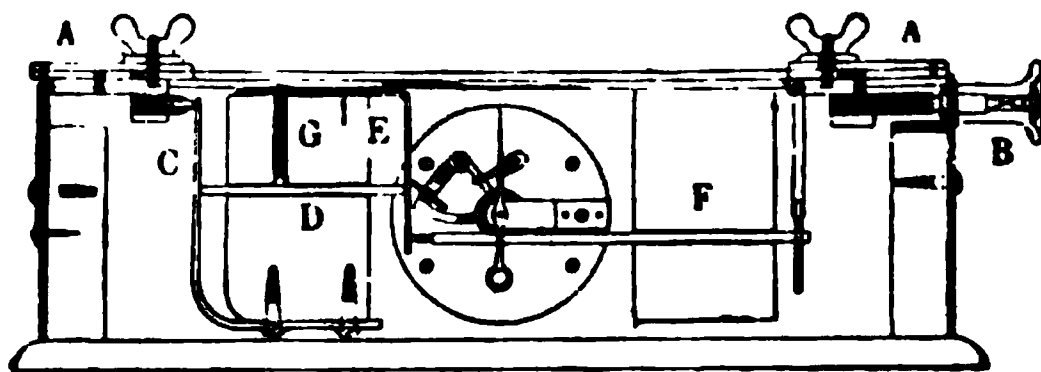
FIG. 75.—Marshall's paper testing machine (secti n).

Resistance to Folding.—The capacity of a paper for resisting wear and tear, folding and crumpling is of considerable importance in high-class papers intended to be frequently handled. The methods in common use are more or less empirical, but nevertheless serve the purpose of comparison.

The loss due to folding may be measured by taking strips in the two directions of the paper, folding them backwards and forwards repeatedly, either by hand or by using a special appliance constructed for this purpose. The strip is then tested in the usual way and the percentage loss of strength calculated.

The resistance to crumpling may be measured by crumpling a sheet of paper between the fingers into a small ball, which is then smoothed out again and the sheet examined for pin-holes produced by the friction. A record is made of the number of· foldings made and the number of holes produced at intervals.

An elaborate machine has been devised for measuring this resistance to crumpling in order to eliminate errors which must occur with empirical methods.

Fig. 76.—Muller's " Bursting Strain " paper testing machine.

Bursting Strain.—There are several machines by means of which the so-called bursting strain of paper is determined. These machines are based upon the application of pressure to the surface of a sheet of paper fixed rigidly between horizontal clamps, the pressure required to burst the paper being registered on an ordinary gauge in terms of pounds per square inch. Machines of this type are very useful for comparative results since no special precautions are necessary in adjusting the paper, whereas in the case of machines for determining tensile strain great care is necessary in proper adjustment, but no measure-

ments of the difference in strength between the machine and cross directions of paper or of elongation under tension are obtained.

Sizing Qualities.—The ink resisting property of a paper may be measured by several methods more or less accurate.

1. *The Use of Strong Ink.*—Lines are drawn on the paper with a soft quill pen and a note made as to the behaviour of the ink. With a paper poorly sized the ink quickly penetrates.

2. *Schluttig and Neumann's Method.*—The paper is fixed on a slip of wood at an angle of 60°. A solution of ferric chloride is allowed to flow down on the surface of the paper and left for 15 or 20 minutes. The paper is then reversed and a solution of nut-gall tannin allowed to flow on the other side of the paper in a direction at right angles to that previously used. Usually 3 lines of solution are produced on each side of the paper. At the points of intersection chemical reaction, more or less definite, between the tannin and iron salt sets in with the formation of a black stain. With well-sized papers the effect may be very slight.

3. *Leonhardi's Method.*—One drop of a 1.5% ferric chloride solution is allowed to fall on the surface of the paper and to remain for a number of seconds equivalent to the weight of the paper in grms. per square metre. Excess of solution is then dried off with blotting-paper and the back of the sheet moistened with a 1% solution of tannin and a note made of the effect produced.

Comparative methods of this description allow of an approximate classification as follows:

1. Unsized,	Drops penetrate quickly.
2. Moderately sized,	Drops require one minute for penetration.
3. Well sized,	Drops require 3 minutes.
4. Very well sized,	Drops require 5 minutes or more.
5. Extremely well sized.	No results.

Absorbency.—This property of paper, viz., its power to absorb water and other liquids is chiefly of importance for blottings, filtering paper and copying paper. The papers are tested by determining the effect produced by suspending strips vertically with the lower end dipping into distilled water, ink or similar solutions. A measurement is made of the rate at which the solution is drawn up by capillary attraction and of the total height to which the solution rises in a given time.

The "zone" test is useful, particularly for blottings. 0.5 c.c.

of ink is cautiously dropped from a burette on to the surface of the paper placed 0.5 in. below the burette in a horizontal position on the top of a beaker. The blot is allowed to dry and it will be found that the blot consists of two zones, the outer one of which is practically impervious to ink as may be tested by passing a pen across from the centre of the blot to the outside.

Fibrous Constituents of Paper.

The vegetable fibres commonly employed for the manufacture of paper are cotton, linen, hemp, esparto, straw, chemical wood pulp, mechanical wood pulp, jute and manila hemp. Other materials, such as ramie, bamboo, rice straw, aloe, and paper mulberry, are occasionally found in paper of foreign origin.

The existence of such fibres and the proportions in which they are present is determined by a microscopic examination. The fibres are identified by their peculiar physical structure, while the application of certain staining reagents serves to assist in differentiating between them, not only by a clearer definition of structure, but by varying colour reactions. The following table shows the staining effect produced by the reagents generally employed.

COLOUR EFFECTS PRODUCED BY STAINING REAGENTS.

Fibres	Iodine solution	Zinc chloride-iodine solution	Magnesium chloride-iodine solution
Cotton, linen, hemp..........	Brown	Wine-red	Reddish-brown
Esparto, straw, bamboo celluloses....................	Grey to greyish-brown	Blue to violet or blue to greyish-violet	Bluish-violet
Wood celluloses..............	Colourless	Blue to bluish-violet	Light brown to red
Manila hemp.................	Grey, brown or yellowish	Dark yellow or greenish-yellow	Yellow, greenish-yellow
Mechanical wood pulp, jute ...	Yellow	Yellow	Yellow
Unbleached manila, straw (partially boiled)................	Yellow	Yellow	Yellow

THE REAGENTS ARE PREPARED AS FOLLOWS:

Ingredients	Iodine solution	Zinc chloride-iodine solution	Magnesium chloride-iodine solution
Iodine	1.15 pts.	1.0 pt.	1.0 pt.
Potassium iodide.............	2.0 pts.	5.0 pts.	5.0 pts.
Water	20.0 pts.	15.0 pts.	20.0 pts.
Zinc chloride	......	40.0 pts.	......
Magnesium chloride	......	......	30.0 pts.

The iodine is dissolved in water with the potassium iodide, and the solution added to the zinc or magnesium chloride. The mixture is allowed to stand and the clear supernatant liquor bottled for use.

Appearance of Fibres Examined by the Microscope.—The physical structure of fibres after they have been boiled, bleached and beaten for the preparation of paper differs considerably in many cases from that of the raw material. That is to say, the processes modify and lessen the characteristic distinctions which are usually pronounced in the raw fibre. The examination of papers containing fibres not commonly used is considerably facilitated by comparison with fibres of known origin. The following is a brief description of the commonly occurring fibres:

Cotton resembles a flat collapsed tube with occasional spiral twists, numerous in the raw material, but less frequent in beaten paper pulp. The cell walls often striated with lattice-like markings. Central canal broad. Complete absence of pores and knots. Ends of fibres blunt.

Linen consists of well-shaped cylindrical tubes with narrow central canal. Ends of fibres pointed. Characteristic knots at intervals in the fibre. Walls of cell marked with pores. In beaten pulp these peculiar features are less marked, the fibre ends being usually frayed out.

Hemp closely resembles linen. Well-beaten hemp is very difficult to distinguish. Cell walls frequently striated parallel to length of fibre, ends flat or fork-shaped. Fibres sometimes flattened out by the beating process will show structure more clearly.

Esparto fibres are fine, slender, short cylindrical tubes with tapered ends and very small central canal. Fibres seldom cut or destroyed by beating. Numerous pear-shaped seed hairs and serrated epidermic cells serve to identify esparto and to distinguish it from straw.

Straw fibres thicker than esparto, which in some respects resembles straw. Central canal irregular in shape. Walls of fibre thickened at intervals, giving appearance of knots. Numerous serrated cells. Many thin transparent parenchyma cells of large area. These latter are entirely absent from esparto cellulose.

Bamboo fibres are long slender tubes with tapered ends. The pulp contains epidermic cells which in general appearance resemble those of esparto and straw, but may be differentiated by careful examination.

Chemical wood pulp fibres differ according to the wood from which the pulp has been made. Generally speaking, fibres are flat and broad and strongly marked with pores and pitted vessels, the shape and number of which serve to identify the species of wood. The fibres from the coniferous woods, such as spruce and fir, are flat ribbons with characteristic pitted vessels while the fibres of the deciduous trees, such as poplar, birch and aspen, are more cylindrical in shape and the pulp contains in addition to the long fibres a number of oval-shaped cells with markings which vary according to the nature of the wood.

Mechanical wood pulp contains fibres of indefinite structure. The fibres of definite shape mixed with bundles of incompletely separated fibres and structureless particles are easily recognised. The use of staining reagents applied to papers containing mechanical wood pulp is of great service in identification.

Jute strongly resembles hemp or manila, having knots and irregular markings. The most characteristic feature is the central canal or lumen which varies in diameter, opening out suddenly in parts and closing up again. Unless completely free from ligneous matter the fibres are found aggregated together in bundles.

Mounting Sample of Paper for Microscope.—0.5 grm. of paper is gently heated in a weak solution of sodium hydroxide to dissolve out the size, well washed and then broken into pulp, either by maceration in a mortar or, better still, by agitation in a bottle with a number of beads. The pulp so produced is washed and a suitable quantity placed on a glass slip, excess of water being drained off by means of blotting paper and one drop of the staining reagent added. The

fibres are carefully distributed by teasing out with a glass rod or a microscope needle, and the cover-glass put on in the usual way.

Percentages of Various Fibres.—When a paper contains several fibres, such as sulphite and mechanical wood pulps in a cheap news, or esparto and chemical wood pulp in a magazine paper, it is necessary to determine the proportions in which the fibres are present. This requires considerable experience which can only be obtained by practising with known mixtures. The simplest method of estimating the percentage of fibre present is to examine 4 or 5 slides, going carefully over the whole of the pulp on the slide and obtaining a "mental impression" of the proportions in which the fibres are present. Some observers count the number of fibres of each kind and estimate the percentage in this way, but the better plan is to become familiar with the fibres by work on papers of known composition so that a correct mental impression is obtained. The microscopic examination of paper in regard to the estimation of the percentages of fibre can only be effected by considerable practice and cannot be accomplished according to any exact methods of analysis.

Mineral Constituents of Paper.—Ordinary papers contain mineral substances technically described as loadings or fillers, added partly for the purpose of giving weight and partly to improve the surface of the sheet and increase its opacity. The substances which may be present in ordinary papers are china clay, barium sulphate of calcium sulphate (pearl hardening, gypsum, terra alba), magnesium silicates (French chalk, talc, asbestine, agalite), satin white (a mixture of pre-cipitated alumina and calcium sulphate).

Percentage of Mineral Substances in Paper.—A convenient weight is ignited in an ordinary crucible until there is no further loss of weight. The ash is weighed and the percentage calculated. Special appliances have been devised for making ash determinations, but these offer no particular advantages.

Nature of Ash in Papers.—In addition to the mineral substances noted papers may contain pigments, such as ultramarine lead chromate, iron oxides and other inorganic bodies, according to the quality of the paper and its special use. All papers contain some ash, which, when in small quantity need not be regarded as evidence of added mineral matter, as it may be derived from the fibrous material, from the alum used for sizing or from hard water.

The complete examination of the ash involves an analysis of a suffi-

cient quantity by ordinary methods which therefore need not be given in detail here.

Sizing Constituents of Paper.—A large number of organic substances have been used for rendering paper more or less impervious to moisture, many of them only experimentally. The common sizing agents are gelatin, starch and rosin, while casein, viscose and algin find a limited use.

Gelatin in paper is easily extracted by gently heating a small sample in water. The extract, cooled and poured into a solution of tannin gives a voluminous flocculent precipitate which shrinks and and coagulates to a small horny-like mass when heated.

The quantitative analysis of paper for gelatin is based on the well-known Kjedahl method. The weighed portion of paper is cut into small pieces (1 or 2 grm.). The amount of nitrogen multiplied by 5.56 gives the weight of gelatin (absolute dry) in the quantity of paper taken for analysis. Pure dry gelatin contains 18.00% nitrogen.

Starch is readily detected by the blue produced when the sample is treated with a weak solution of iodine. Quantitative estimation are based on:

1. Extraction with suitable solvents. A weighed quantity of paper dried at 100° is heated with absolute alcohol containing a few drops of hydrochloric acid to remove resinous substances, the amount of which is determined by loss of weight. The further loss sustained by boiling with a mixture of water and rectified spirits containing a few drops of acid is attributed to the removal of starch.

2. Conversion of starch into dextrose by treating a known weight of paper with a weak solution of sulphuric acid and estimation of the dextrose by means of Fehling's solution.

Rosin.—The paper cut up into small strips is heated in a test-tube with absolute alcohol containing a few drops of acetic acid. The extract when poured into water gives a turbid solution more or less intense according to the percentage of rosin present in the paper.

A little ether poured on the surface of the paper will dissolve the rosin present, which, as the ether evaporates, forms a brownish ring.

A strip of paper sized with rosin if partially immersed in concentrated sulphuric acid develops a reddish tint at the edge in contact with the surface of the acid. There must be no mechanical wood pulp present in paper submitted to this test.

The amount of rosin size in paper is estimated by the loss in weight caused by extraction with absolute alcohol containing a few drops of acetic acid.

The proportion of rosin may also be determined approximately by comparing the turbidity of the extract when poured into water with that produced by adding a known volume of a 1% solution of rosin in absolute alcohol to an equal volume of distilled water.

Casein.—Paper having a strong alkaline reaction when tested with litmus probably contains casein as the sizing agent. This is chiefly used with the so-called "art papers." The casein in paper is extracted by boiling with sodium carbonate or ammonium hydroxide. The extract is neutralised with acetic acid, evaporated to a small bulk and treated with a mixture of 1 part strong sulphuric acid and 2 parts of acetic acid. A reddish-violet is obtained which indicates casein. Gelatin does not give such a pronounced colour. The proportion of casein present is determined by the Kjedahl process.

Impurities in paper consist of fibrous and other ingredients which affect the physical properties, and soluble constituents which affect its colour and durability.

Fibres.—Occasionally papers are contaminated with extraneous fibres which impair its strength. Common newspapers frequently break on the printing machine owing to the presence of undigested fibres of coarse wood pulp or jute. The latter is frequently derived from string or canvas used in packing wood pulp. The nature of such fibres is determined microscopically.

Particles of dirt will be found in paper and may be traced, chiefly by microscopic examination, to coal, coke, coarse imperfectly ground loading, traces of stone from the grindstones used for the manufacture of mechanical pulp, specks of iron and other metals, minute pieces of rubber and similar substances.

Transparent spots in paper are usually traceable to rosin derived either from the sulphite wood pulp used in the paper or to imperfectly boiled rosin size. Impurities of this character can generally be removed by extraction with absolute alcohol and ether. Sometimes the transparent spots are due to the crushing of the fibre by the calender rolls used in imparting a surface to the paper. If a thick place in the sheet is due to the aggregation of a number of fibres, the pressure of the calenders will render the spot transparent.

Soluble Constituents.—In the process of manufacture certain impurities may be left in the pulp such as:

Acid.—The presence of free acid in paper cannot be determined with ordinary litmus, since alum used in sizing gives an acid reaction with litmus paper. The aqueous extract should be tested with methyl orange and Congo red.

Sulphides, usually traceable to chemical wood pulp, may be detected by boiling the paper in a dilute solution of acid and allowing the steam to impinge on the surface of a filter-paper impregnated with lead acetate.

Iron.—Traces of iron salts tend to lower the colour of ordinary paper and are, of course, undesirable in photographic papers. The usual tests for iron can be applied.

Examination of Special Papers.—The analysis of papers manufactured for special purposes is governed largely by a knowledge of the methods of manufacture or a knowledge of the uses to which the paper is put. . Of this we quote a few examples.

Tinfoil.—The amount of tin on the surface of the paper is ascertained by removal of the tin with acid and subsequent examination of the solution.

Gilt and bronze papers are frequently examined for the nature of the material used in gilding. The substance is dissolved in acid and the solution examined in the ordinary way. Most of the matter used for gilding consists of copper and its alloys.

Vulcanised Fibre.—This is examined microscopically in order to determine the extent of the treatment with zinc chloride and any traces of zinc can be found by examination of the ash of the paper.

Coated Papers.—The heavy coating on so-called art and other surfaced papers, being a mixture of some inert mineral substance, such as china clay or barytes, mixed with an adhesive, such as glue or casein, can be removed by cautious treatment in hot water or in a weak solution of sodium carbonate. A sheet of paper of known area is weighed, soaked in hot water or alkali and the coating removed by cautious rubbing with a camel's-hair brush. The "body" paper, so-called, is then dried, and the loss in weight is a measure of the amount of coating substance used. The percentage of gelatin or casein can be estimated in the original coated paper by the Kjedahl process.

Waxed papers can be treated with suitable solvents to remove the wax with which the paper is impregnated.

Waterproof Papers.—Extraction with alcohol and ether will re-

move lac, rosin and similar products employed for the purpose of making paper waterproof. Borax, alum, glue and metallic oxides may be sought for in papers of this class.

Cheque Papers.—Iron ferrocyanides mixed with other ferrocyanides, together with potassium and sodium iodides, are frequently used in papers of this class and may be found in the aqueous extract of the paper or by applying suitable reagents to the surface of the paper. Other salts may be found by careful examination of the aqueous extract.

WOOD PULP.

Mechanical Wood Pulp.—The quality of this material is determined chiefly by its freedom from coarse heavy chips known as shives. These are slivers of pulp produced during the grinding of the wood which have not been properly removed by the strainers. The proportion present in a sample of pulp may be found by macerating the latter in a mortar, agitating the mass in a large quantity of water, and separating the shives by a process of elutriation. Mechanical pulp should not be contaminated with jute fibres from the canvas used in manufacture nor with particles of stone from the grindstones. The presence of mechanical pulp in paper is readily detected by the intense yellow produced when a sheet of the paper is moistened with a 4% solution of aniline sulphate.

Another useful reagent is phloroglucinol, which imparts a reddish tint to paper containing mechanical pulp, the depth being proportional to the amount present. The reagent is prepared by dissolving 2 grm. of phloroglucinol in 50 c.c. absolute alcohol and adding 25 c.c. of hydrochloric acid. It should be noted that papers coloured with metanil yellow are instantly turned to a reddish shade by the acid in the reagent, and this effect must not be confused with the mechanical pulp reaction.

The proportion of mechanical wood in a paper is judged by this colour test or by a microscopical examination. A method has recently been introduced for estimating the amount by a volumetric process. It depends on the absorption of the phloroglucinol by the ligno-cellulose of the pulp and a titration of the unabsorbed reagent by formaldehyde.

Chemical Pulp.—Wood cellulose is isolated by 3 processes: treatment with calcium hydrogen sulphite (bisulphite) or with sodium

Fig. 77.—Straw pulp; small cells from washings

Fig. 78.—Manila hemp.

Fig 79.—Mechanical wood pulp (ground wood).

Fig. 80.—Esparto pulp.

Fig 81.—Poplar pulp.

Fig. 82.—Cotton pulp.
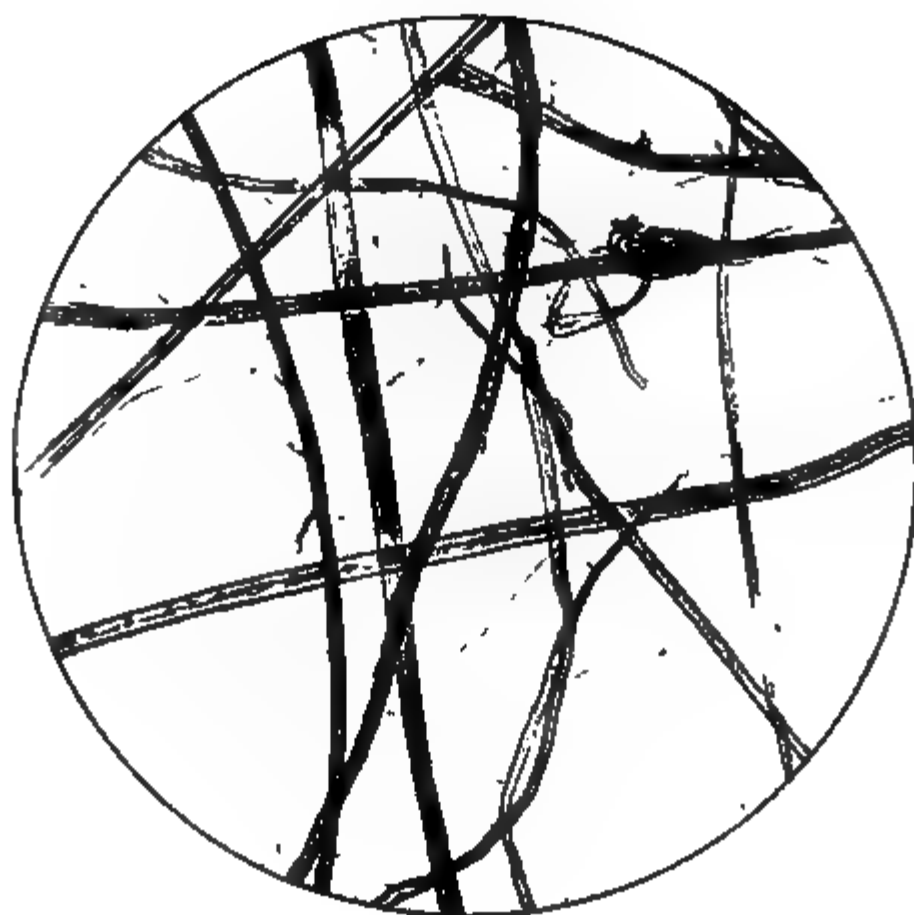
FIG. 83.—Linen pulp.

FIG. 84.—Bamboo pulp.

FIG. 85 —Spruce pulp.

hydroxide or with a mixture of sodium hydroxide and sodium sulphide. These pulps are usually described as sulphite, soda and sulphate pulps. The last two are for all practical purposes the same and are readily distinguished in their commercial forms from the sulphite by the difference in feel, bulk and texture. The chemical differences are very slight, the sulphite pulp containing 0.5% resin in contrast with soda pulps which contain 0.05%. Slight differences also in behaviour towards certain aniline dyes, such as malachite green and saffranin, are used for distinguishing the fibres when examined microscopically, but the reactions are not of a very definite character. It is therefore difficult to determine the proportions present in a paper which contains both classes of pulp and at present no exact methods are known by which the complete absence of a soda pulp in a paper presumed to be all sulphite, or vice versa, can be ascertained.

Moisture in Wood pulp.—The pulp used for paper-making is supplied in the condition of dry sheets or of moist sheets containing 50% of air-dry pulp. The air-dry pulp is calculated on the basis of 10% moisture; that is, 90 parts of absolute dry pulp (dried at 100°) is equivalent to 100 parts of air-dry pulp. All supplies are tested for moisture.

According to regulations agreed to by pulp manufacturers and paper-makers, not less than 2% and not more than 4% of the number of bales in a consignment are selected, such bales being sound, intact and representative. From each bale, when weighed, 3 or 5 sheets are drawn and sampled by what is known as the "wedge" system. A wedge having its apex at the centre of the sheet and its base at the outer edge mathematically constitutes a fair sample of the sheet. The wedges are cut with bases of varying width if necessary in order to obtain samples that correspond with the volume of pulp they are supposed to represent.

The sample of pulp is weighed, dried at 100° in a water-oven, and the absolute dry weight determined.

The usual form of British certificate is as follows:

WOOD PULP MOISTURE CERTIFICATE.

(Form adopted by the British Wood Pulp Association.)

This is to Certify that I have tested for moisture a parcel of **Moist Mechanical** Pulp, said to consist of 146 bales, marked **"Star" ex s.s. "Norway"** lying at Messrs. **The Hampden Paper Mills, Newton.** The sample were drawn by me on

	Bales	T.	cwt.	qrs.	lbs.
Total gross weight of bales sampled (intact)	6	1	3	1	18
(For numbers and detailed weights see below.)					
Weight of Parcel calculated from above	146	28	9	2	18
Percentage of absolutely dry pulp in the sample ...			46.22 per cent.		
" moisture in the sample			53.78	"	
" air-dry or moist pulp in the parcel on the basis of					
90 = 100 (air-dry)			51.35	"	
45 = 100 (moist)			102.70	"	
" excess Moisture, Fibre					

		T.	cwt.	qrs.	lbs.
Weight of Pulp to be invoiced	{ Air-dry ...	14	12	2	1
	{ 50% Moist	29	5	0	2

NUMBERS AND DETAILED WEIGHTS OF BALES SAMPLED.

Cwt.	qrs.	lbs.
3	3	16
3	3	16
3	3	24
3	3	11
3	3	19
3	3	16
3	1	18
23		

Analyst.

The following is the usual form of the American certificate:

Moisture @ 100° C.,...................

Bone-dry pulp, ...

Air-dry pulp, on 10% basis.............

of the above bales, carefully selected, (weighing   lbs.) were opened, and an outside, an intermediate, and an inner sheet were taken from each: portions of all these sheets were kept in an air-tight vessel for the above analysis.

The Bleaching of Wood Pulp.—The amount of bleaching powder required to bleach sulphite, soda and sulphate wood and other half stuffs is an important factor. The percentage consumption varies with different pulps.

Esparto,	10–15%.
Soda wood,	18–25%.
Sulphite wood,	9–20%.

From the carefully selected representative sample, a small quantity of 5 or 10 grm. is accurately weighed out and macerated with a little warm water in a mortar so as to thoroughly break up the pulp into a fibrous condition. The moist pulp is transferred to a convenient sized beaker or jar and a clear solution of chloride of lime added, the quantity being determined by the nature of the fibre. It is best to add liquor containing available chlorine equivalent to 25% of bleaching powder calculated on the air-dry weight of pulp. The mixture is kept for 2 or 3 hours at a temperature of 38° C. to 40° C., being frequently stirred until the colour is white. If insufficient bleach liquor has been added, the chlorine may be exhausted before the pulp is white, as indicated by a starch-iodide test-paper. When the colour is satisfactory, the liquor is filtered off and titrated with decinormal arsenic solution in order to determine the amount of bleach unconsumed. If a considerable excess of bleach has been used in the first experiment, the test should be repeated, using only a slight excess of clear bleach liquor over and above the amount shown to be consumed in the first. Example:

Decinormal arsenic solution, 1 c.c. = 0.00355 grm. chlorine
                                    = 0.01 grm. normal bleaching powder.
Bleach liquor used,   1 c.c. = 3.8 c.c. arsenic
                                    = 0.038 grms. normal bleaching powder
Pulp taken, 10 grms.
Bleaching powder added, 2 grms.
    in the form of solution, 52.62 c.c.
Arsenic required to neutralise unconsumed bleach, 7.6 c.c.

Bleaching solution actually used, 50.62 c.c.

= 1.923 grms. powder

Bleaching powder consumed by air-dry pulp = 19.2%.

Examination of New Fibres for Paper-making.—The scheme of analysis generally adopted for plant substances is that devised by Cross and Bevan, which is shown in the following schedule:

Moisture,	Hygroscopic water, or water of condition. Loss in drying at 100°.
Fat, wax and resin,	By extraction with solvents.
Ash,	Residue left on ignition.
Hydrolysis (a),	Loss of weight on boiling 5 minutes in 1% solution of sodium hydroxide.
(b),	Loss of weight on continuing to boil one hour
Cellulose,	Boiling with weak alkali, exposure of washed product to chlorine gas, and heating in solution of sodium sulphite. Final immersion in sodium hyposulphite.
Mercerising,	Loss of weight on treating 1 hour with 15 to 25% solution sodium hydroxide.
Nitration,	Weight of nitrated product obtained by treatment with mixture of equal volumes of nitric and sulphuric acids one hour in cold.
Acid purification,	Loss of weight after boiling with 20% acetic acid and and washing with water and alcohol.
Carbon percentage,	Combustion with chromic acid after solution in sulphuric acid.

Yield of Paper-making Fibre.—The suitability of a new material is best determined by treatment of 500 to 1000 grm. The fibre previously cut up into small pieces is packed closely in an autoclave, covered with sodium hydroxide solution of known density, strength, and boiled for 6 or 7 hours at a definite pressure. For a first trial the alkali solution should have a sp. gr. of 1.050 and the pressure should not exceed 60 pounds per square in. These conditions will be modified in the succeeding trials according to the results of the first. The resultant pulp is washed and immersed in a known volume of bleach liquor for 2 or 3 hours at 38°. The bleached pulp is removed, and any available chlorine still remaining in the residual liquor estimated by means of standard arsenical solution.

The pulp is washed thoroughly and made up into small sheets for convenience. The fibre of the pulp is examined microscopically and a record made of the dimensions and general characteristics.

The total yield of paper-making fibre from the plant substance is, of course, easily obtained by this experiment. The sodium hydroxide used is ascertained by titration of the residual liquor from the digester. In actual practice, the solution must be sufficient in quantity to cover the fibre and of sufficient strength to effect the isolation of the cellulose. Hence a considerable excess is always necessary.

ACID DERIVATIVES OF ALCOHOLS.[1]

(VEGETABLE ACIDS).

By HENRY LEFFMANN.

These acids are numerous and form well-marked series of salts. In concentrated form they are mostly actively corrosive, and even when considerably diluted with water produce strongly acid liquids which affect nearly all indicators and dissolve oxides and carbonates; a few of them dissolve such metals as iron and zinc. Many of them are volatile at ordinary temperatures; others, that are not so readily volatile, distil with open steam; some distil only with superheated steam. The salts are mostly colourless unless formed with colour-producing metals, and are usually without odour, but the liability of many of them to hydrolyse slightly often causes the apparently pure salt to have the odour of the acid from which it was produced. All the salts are decomposed by heating, generally leaving a residue of carbonate with free carbon, but when an easily reducible metal is present, the residue may be either an oxid or the free element.

The following table shows the manner in which the neutral solutions of the potassium or sodium salts of the acids of this division are affected by cold neutral solutions of barium, calcium and ferric chlorides, lead acetate and silver nitrate. The reactions refer to moderately concentrated solutions of the salts. When the precipitate is somewhat soluble in water, so as to render its production uncertain, the letter P is placed within parentheses. S signifies that the substance formed is soluble, and hence that no precipitate is obtained. Except when otherwise mentioned, the precipitates are white. In addition to the reactions with the above metallic solutions, columns are added showing the reactions of the organic acids with other important reagents. R signifies "reduction" and o "no effect":

TABLE SHOWING THE REACTIONS OF THE SALTS OF SOME OF THE VEGETABLE ACIDS.

Name of salt in solution	With barium chloride	With calcium chloride	With ferric chloride	With lead acetate	With silver nitrate	With hot Fehling's solution	With permanganate in cold acid solution	With hot concentrated sulphuric acid	Remarks
Acetate,	S	S	Red	S	(P)	o		Odour of acetic acid.	Silver salt not reduced on heating solution.
Formate,	S	S	Red	S	(S)	..	R	Carbon monoxide evolved.	Silver salt or solution reduced on heating.
Oxalate,	P	P	S	P	P	o	R	Carbon monoxide and dioxide evolved.	A yellow precipitate sometimes occurs on adding ferric chloride.
Lactate,	S	S	S	S	S	o	R	Carbon monoxide evolved. Brown colour.	See Lactic acid.
Succinate,	(P)	(S)	Red-brown precipitate.	P	P	o	o	No change	Barium and calcium salts precipitated on adding alcohol.
Malate,	S	(S)	S	P	P	o	R	Darkened.	Calcium salts insoluble in dilute alcohol.
Tartrate,	P	P	S	P	P	o	R	Charring.	Silver salt reduced on heating.
Citrate,	P	P	S	P	P	o	o	Carbon monoxide evolved. Brown.	Calcium salt precipitated on boiling and redissolved on cooling.
Aconitate,	(P)	(P)	..	P	P	..	..	..	Calcium salt is soluble in 100 parts of water.
Meconate,	(P)	P	Red	P	P	..	..	..	Action of oxidising agents not recorded.

Colour Reactions for Organic Acids.—Some phenolic derivatives give colours with these acids. Messrs. H. J. H. Fenton and G. Barr have made a number of comparative experiments. They find that not only the acids, but their salts and esters can be detected even in minute amount by the reagents they used. The substance to be tested is mixed with strong sulphuric acid and the reagent. In testing dry material it is necessary to add a drop of water before deciding upon

the result. The following is an abstract of the method (*Proc. Cambridge Philosoph. Soc.*, 1907, **14**, 386). In the table, A indicates the result on adding ammonium hydroxide after the reagents have been allowed to act for a short time. The changes are sometimes slow. Many acids were tried. The following, among others, gave no characteristic results: Tartaric, citric, suberic, sebacic, mucic, malic, succinic, malonic, hippuric, acetic, butyric, stearic, aminoacetic, cinnamic.

Acid	Resorcinol	Phenol	Pyrogallol	1-2 Cresol
Formic	Strong orange-red changing to blood-red. A, to dilute solution, green becoming purple.	Pink.	Pink, passing to orange-red and scarlet.	Light red.
Oxalic	Slight yellow, passing to dark blue. A, change to pink.	Faint pink passing to red.	Dirty green passing to orange. A, changes to intense blue, then purplish-brown.	Crimson. A, gives purple.
Lactic	Yellow passing to orange. A, gives fluorescent green.	Yellow or orange.	Orange.	Red-brown.

The following special methods with resorcinol are given by Mulliken (*Identif. Pure Org. Comps.*, Vol. I):

A small amount of the acid is mixed with a few drops of a freshly-prepared solution of resorcinol, the dish placed on the water-bath and at intervals of half a minute portions of the liquid are removed and the depth of colour noted. When the maximum is reached, the liquid is diluted cautiously with water and the colour changes noted.

Citric acid, pale greenish-blue, changing blue-green, then to pale impure green. Colour after dilution much paler.

Tartaric acid, pale blue-green for a moment, then pure intense green. Dilution with water gives orange-yellow.

Malic acid, momentary greenish-yellow changing to intense yellow, which is permanent. Dilution gives orange-yellow more intense than from the other two acids.

Acetic Acid.

This occurs in some plants and is a frequent product in chemical reactions. It is produced by the acetic fermentation of sugar and by the limited oxidation of alcohol. A large quantity is obtained by the distillation of wood. The crude material from this source is usually termed "pyroligneous acid."

Acetic acid is a colourless liquid, strongly acid and pungent. It crystallises in transparent plates, melting at 16.7°, and hence is often termed "glacial acetic acid." Acetic acid remains liquid if cooled in a closed vessel, even below 0°, but on opening or shaking the vessel or dropping in a fragment of the solid acid, the whole solidifies and the temperature rises to 16.7°. A small addition of water lowers the m.p. of acetic acid very considerably, so that an acid containing 13% of water melts below 0°, and one containing 38% of water (corresponding to $C_2H_4O_2 + 2H_2O$) has a m.p. of −24°. More water raises the m. p.

Acetic acid boils at 119° and distills unchanged. In distilling hydrated acid the last fractions are absolute or nearly so.

Addition of water to acetic acid causes evolution of heat, and contraction in volume until the mixture contains about 23% of water, Acid of this strength has a higher sp. gr. than the glacial acid, so that either concentration or dilution causes a diminution. The sp. gr. of moderately concentrated solutions of acetic cannot be used in ascertaining their strength, but is of service in examining the dilute solutions.

The table on page 489 taken from the United States Pharmacopœia, 1900–05 (8th decennial revision) shows the sp. gr. of acetic acid of different strengths, all figures being at 15°/15°. It will be seen by the table that acid of 100% and acid of approximately 43% will coincide in gravity.

Absolute acetic acid is miscible in all proportions with water, alcohol and ether. It is powerfully corrosive, dissolves many essential oils, camphor and resins, phenols, gelatin and many metallic salts insoluble in water. The liquid acid is not inflammable, but the vapour burns with a blue flame.

Acetic acid is stable. The most powerful oxidising agents attack it with difficulty. Chromic acid has no effect on it; a solution of chromic acid in acetic acid is employed for the oxidation of hydrocarbons. Nitric acid has no action; chlorine converts it into chloracetic acid.

Per cent.	Sp. gr.	Per cent.	Sp. gr.	Per cent.	Sp. gr.
1	1.0015	19	1.0278	37	1.0500
2	1.0030	20	1.0292	38	1.0510
3	1.0045	21	1.0306	39	1.0521
4	1.0060	22	1.0319	40	1.0531
5	1.0075	23	1.0332	45	1.0579
6	1.0090	24	1.0345	50	1.0623
7	1.0105	25	1.0358	55	1.0661
8	1.0120	26	1.0371	60	1.0693
9	1.0135	27	1.0384	65	1.0720
10	1.0150	28	1.0396	70	1.0741
11	1.0165	29	1.0408	75	1.0754
12	1.0179	30	1.0420	80	1.0756
13	1.0193	31	1.0432	85	1.0747
14	1.0208	32	1.0444	90	1.0721
15	1.0222	33	1.0455	95	1.0668
16	1.0236	34	1.0467	100	1.0562
17	1.0250	35	1.0478		
18	1.0264	36	1.0489		

Detection of Acetic Acid and Acetates.—Most of the acetates are soluble in water. A few oxyacetates ("basic" acetates) are insoluble; silver and mercurous acetates are sparingly soluble. Hence, acetic acid cannot be estimated or readily detected by precipitation. Free acetic acid may generally be recognised by its odour and other physical properties, or it may be neutralised by sodium hydroxide and examined by the following tests:

Metallic acetates give the following reactions:

Subjected to dry distillation, acetone, is given off, having a highly characteristic odour.

Heated in the solid state in admixture with arsenous oxide (As_2O_3), acetates give an alliaceous and very characteristic odour of kakodylic oxide. Only a very minute amount of materials should be used in this test, as the products are very poisonous.

Heated with sulphuric or phosphoric acid, acetic acid is evolved.

Heated with alcohol and concentrated sulphuric acid, the fragant and characteristic ethyl acetate (acetic ether) is produced.

The neutral solution, on treatment with ferric nitrate or ferric chloride, avoiding excess, gives a deep red liquid containing ferric acetate This is decomposed on boiling, the liquid becoming colourless and depositing reddish-brown ferric oxyacetate. The reaction is imperfect if the iron solution is added in excess. The cold red liquid is not

decolourised on addition of mercuric chloride (distinction between acetates and thiocyanates); and is not taken up by ether on agitation (distinction from thiocyanates); but the colour is readily destroyed on addition of cold dilute sulphuric or hydrochloric acid (distinction from meconates).

Insoluble (basic) acetates may be converted into sodium acetate by boiling with sodium carbonate and filtering off the insoluble carbonate.

Acetates containing nitrogenous bases respond, as a rule, to the foregoing tests, but the acetic esters do not. The latter can, however, be saponified by alcoholic alkali (see page 232), and after distilling off the alcohol the acetate can be examined.

Assay of Acetic Acid and Acetates.—For samples consisting only of acetic acid and water the sp. gr. will often furnish sufficient information or the liquid may be titrated.

Phenolphthalein is applicable as indicator, sodium acetate being neutral to it, but alkaline to litmus. The end-reaction is sharp. Highly-coloured liquids, such as vinegar, may be largely diluted before titrating, as the delicacy of the reaction is but little diminished.

Methyl-orange and phenacetolin are not suitable indicators for titrating acetic acid.

Acetates containing metals of the alkalies and alkaline earths are converted into carbonates on ignition. In many cases the amount of acetate originally present may be ascertained by titrating with standard acid, the residue of the ignition. Each c.c. of normal acid required for neutralisation represents 0.060 grm. of acetic acid in the sample.

Salts of metals completely precipitated by sodium carbonate (e. g., calcium, lead, iron) may be decomposed by a known quantity of it, the liquid well boiled, filtered, and the filtrate titrated with standard acid. The loss of alkalinity represents the acetic acid originally present as an acetate. Before adding the sodium carbonate the solution must be neutral.

In presence of salts of inorganic acids, the last method is valueless, but a modification may be employed: The excess of sodium carb nate is neutralised by hydrochloric acid, the liquid evaporated to dryness, the residue gently ignited, and the resultant carbonate titrated with standard acid. Each c.c. of standard acid used represents 0.060 grm. of acetic acid. Other organic acids that may be present will be included as acetic acid.

Free acetic acid may also be determined by adding excess of pure precipitated barium carbonate to the solution. The liquid is well boiled, filtered, and the barium in the filtrate precipitated by dilute sulphuric acid. 233 parts of precipitate obtained represent 120 of acetic acid in the sample taken. This process is applicable in presence of oxalic, phosphoric, sulphuric and other *free* acids forming insoluble barium salts, but is useless in presence of soluble oxalates, phosphates and sulphates. The method is available in presence of alkaline chlorides, but not in presence of free hydrochloric acid, unless the solution is previously treated with excess of silver sulphate. Acetates and chlorides of metals of the alkalies and the alkaline earths do not interfere, but acetates and other salts of iron, aluminum and other metals precipitable by barium carbonate must be absent.

The estimation of acetic acid in acetates is best effected by distilling the salt to dryness with a moderate excess of sulphuric acid or with acid sodium sulphate. Water should then be added to the contents of the retort and the distillation repeated. A third, and even a fourth distillation will sometimes be necessary, as the last traces of acetic acid are volatilised with difficulty.

In presence of chlorides, excess of silver sulphate should be added before commencing the distillation.

In presence of sugar or other bodies liable to decomposition by sulphuric acid, phosphoric acid should be substituted . Care should be taken that the phosphoric acid used is free from nitric and other volatile acids. This is best insured by adding a little ammonia and heating the acid to fusion in a platinum crucible.

For the estimation of acetic acid in presence of its homologues, see the analysis of calcium acetate.

Pyroligneous Acid.—Pyroligneous acid or wood vinegar is the crude acetic acid obtained by the distillation of wood. It is a very complex product, containing, among other substances, homologues of acetic acid from formic to caproic acid; crotonic and angelic acids; furfural; bodies of indefinite nature called "wood-oils"; pyrocatechol; acetone and other ketones of the acetic and oleic series; methyl alcohol and the other constituents of wood-spirit. By neutralising the crude product with lime and distilling, the volatile substances of indifferent nature are removed. When partially concentrated, the solution is faintly acidulated with hydrochloric acid, when creasote and various tarry matters separate out; and the clear liquid on evaporation to dry-

ness yields a brownish residue, which is heated to about 230° to de compose the empyreumatic products. On distillation with hydrochloric acid a comparatively pure acid may be obtained, which can be further purified by rectification with a little potassium dichromate. A better product is said to be obtainable by converting the acid into a sodium salt, heating to destroy tarry matters and distilling with hydrochloric or sulphuric acid.

The empyreumatic odour of acetic acid derived from the dry distillation of wood is in great measure due to furfural, vapours of which are always produced if a warm mixture of sulphuric acid and water is poured on bran or sawdust, or if bran is distilled with an equal weight of sulphuric acid and three parts of water. If the vapours of furfural are evolved in a beaker covered with filter-paper soaked in aniline, the latter will turn red, but this soon disappears. This reaction may be employed for the detection of furfural which may be removed from pyroligneous acid by agitating the liquid with 3% by volume of benzene.

Pyroligneous acid differs much in strength according to the kind and state of division of the wood used for distillation, and is also affected by the construction of the retorts. Lopwood yields stronger acid and less tarry and resinous matters than spent dye-woods and sawdust, even though of the same kind.

Pyroligneous acid from finely-divided wood has a sp. gr. of 1.040 to 1.045, and contains, on an average, about 4.5% of acetic acid. The product of the distillation of lop-timber contains an average of 7.75% of real acid.

The strength of pyroligneous acid may be ascertained by titration with standard alkali and phenolphthalein, but the liquid is frequently too dark in colour to permit of the end-reaction being readily observed. Calcium and sodium sulphates and acetates are frequently present. In the absence of sulphates, pyroligneous acid is best assayed by treatment with excess of barium carbonate, with estimation of the dissolved barium as sulphate.

Commercial acetic acid ranges in strength from the nearly absolute glacial acid to the weakest vinegar. The proportion of real acetic acid may be ascertained by the methods already described: in certain cases by the sp. gr.; and in the case of glacial acid by the solidifying point.

The assay of glacial acetic acid, pyroligneous acid and vinegar is described in the respective sections treating of these products.

Commercial acetic acid is often prepared by distilling sodium or calcium acetate with sulphuric or hydrochloric acid. It is liable to contain the following impurities:

Sulphuric acid and sulphates, indicated and estimated by addition of barium chloride, which in their presence throws down white barium sulphate.

Sulphurous acid, indicated by adding barium chloride in excess, filtering from any precipitate, and adding bromine water to the clear .filtrate. An additional precipitate of ·barium sulphate indicates the previous presence of sulphurous acid, and from its weight the amount of impurity can be calculated.

Hydrochloric acid and chlorides, detected and estimated by addition of silver nitrate.

Copper and lead, detected by evaporating a considerable bulk of the sample to a small volume, diluting with water, adding a few drops of hydrochloric acid, and passing in hydrogen sulphide which produces a black or brown colouration or precipitate in presence of lead or copper. If much organic matter is present, the evaporation should be carried to dryness and the residue ignited in porcelain. The heavy metals are then sought for in the residue in the manner described on page 63. A delicate test for copper is the red-brown precipitate or colouration produced by potassium ferrocyanide in the original liquid, or the same concentrated and then diluted with water. If iron is present in such quantity as to give a blue precipitate and thus interfere with the reaction, it must first be removed by addition of bromine water and excess of ammonia, and copper sought for in the filtrate after acidifying with acetic or hydrochloric acid. Samples of pickles suspected to be coloured with copper should be moistened with sulphuric acid, ignited, and the ash dissolved in nitric acid, and tested in acid solution with potassium ferrocyanide, after separation of the iron and phosphates with ammonia. The copper can be determined by electrodeposition on the inside of a platinum crucible by an electric current. *Tin* and *zinc* have been occasionally met with in acetic acid and vinegar.

Salts of calcium are detected by partially neutralising the solution with ammonia and adding ammonium oxalate, which will produce a white precipitate of calcium oxalate.

Empyreumatic and indefinite organic bodies may be detected by exactly neutralising the acid with sodium carbonate and tasting and

smelling the warmed liquid.   The neutralised acid gives a precipitate
when heated to boiling with ammonio-silver nitrate, and the original
acid darkens when heated to boiling with an equal measure of concen-
trated sulphuric acid, if the above impurities are present.   A com-
parative estimate of the proportion of empyreumatic impurities pres-
ent may be made by diluting 10 c.c. of the sample to 400 c.c. with
water, adding hydrochloric acid, and titrating with permanganate till
the pink colour is permanent for 1 minute.

Formic acid frequently occur's in acetic acid.   The estimation of it
has been investigated by H. Ost and F. Klein (*Chem. Zeit.* 1908, **32,**
815), who compared several processes, such as neutralizing with
alkali and titrating with permanganate; oxidising with standard
chromic acid and titrating for the excess of this acid; treating with
mercuric chloride and weighing the separated metal.   These methods
are fairly accordant, and probably in absence of substances (other
than formic acid) capable of reducing permanganate, the per-
manganate method is the best.   (See also pp. 520 and 521).   Ost and
Klein found somewhat over 0.5% formic acid in some samples.   This
cannot be removed completely by distillation or by direct action of
potassium permanganate on the acid, but is best removed by crystal-
lization.

General fixed impurities are detected and estimated by evaporating
of a known measure of the sample to dryness and weighing the
residue.

Glacial acetic acid (absolute acetic acid).   The properties of
this substance have been already described.

Commercial glacial acetic acid should contain at least 97% of the
absolute acid.   This may be ascertained by agitating 1 volume of the
sample with 9 of oil of turpentine.   Complete solution occurs if the
strength is 97% or above.   Samples containing 99.5% of absolute acid
are miscible with oil of turpentine in all proportions.   Oil of lemon
if freshly distilled, may be employed instead of turpentine.

A more delicate test for water is to treat the sample in a dry test-
tube with an equal measure of carbon disulphide, and warm the mix-
tnre in the hand for a few minutes.   The liquid will be turbid if any
water is present in the sample.

The influence of water on the m.p. of glacial acetic acid is shown
in the following table by Rudorff (*Pharm. Jour.* [3], 1872, **2,** 241):

Solidifying point. °C.	Water to 100 parts of real $C_2H_4O_2$.	Solidifying point. °C.	Water to 100 parts of real $C_2H_4O_2$.
+16.70	0.0	6.25	8.0
16.65	0.5	5.30	9.0
14.80	1.0	4.30	10.0
14.00	1.5	3.60	11.0
13.25	2.0	2.70	12.0
11.95	3.0	—0.20	15.0
10.50	4.0	—2.60	18.0
9.40	5.0	—5.10	21.0
8.20	6.0	—7.40	24.0
7.10	7.0		

The strength of glacial acetic acid may also be ascertained as on page 490. The sp. gr. is not an indication of value. Impurities may be sought for as on page 502.

Vinegar.

Properly speaking, vinegar is a more or less coloured liquid, consisting essentially of dilute acetic acid, obtained by the oxidation of alcohol. Sometimes the term is improperly extended to pyroligneous acid, or "wood vinegar," while acetic acid is called "distilled vinegar." In the United States, vinegar made by oxidising dilute alcohol 's often called "spirit" vinegar, and as the dilute alcohol is sometimes called "low wines" the vinegar is called "wine" vinegar, but such a misleading name is now generally forbidden by laws against misbranding.

The reaction between alcohol and oxygen takes place under the influence of platinum-black and some other bodies, but the formation of vinegar from alcoholic liquids usually depends on microorganisms. Mechanical arrangements are employed to expose a large surface of the alcoholic liquid to the air, so as to diminish the time required for acetification.

Besides acetic acid, vinegar often normally contains more or less of other organic acids, sugar, dextrin and colouring matters. The agreeable aromatic smell is doubtless due to esters, and is sometimes imitated by direct addition of ethyl acetate.

The sp. gr. of vinegar is of no value as an indication of its strength in acetic acid, as the proportion of extractive matter differs much in

vinegar from various sources. The "proof vinegar" of the (British) Excise contains about 5% of acetic anhydride, or 6% of the absolute acid, and has a sp. gr. of 1.019. By the manufacturer, vinegars of different strengths are distinguished by the number of grains of pure dry sodium carbonate required for the neutralisation of 1 fluid ounce. Thus "proof vinegar" is known as "No. 24," from the fact that 24 grains are required for the neutralisation of 1 ounce. The weaker qualities are Nos. 22, 20 and 18. As 60 grains of absolute acetic acid, or 51 of acetic anhydride, are neutralised by 53 of sodium carbonate, the number of grains of the real acid contained in each fluid ounce of the vinegar can be ascertained by multiplying the "number" of the sample by $\frac{6.0}{5.3} = 1.132$. If the "number" is multiplied by the factor 0.259, the product will be the parts by weight of absolute acid in 100 measures of vinegar.

Genuine vinegar of good quality will not contain much less than 5% of absolute acetic acid, though something depends on the origin of the vinegar, cider-vinegar being naturally the weakest and wine-vinegar the strongest in acetic acid.

The proportion of acetic acid in vinegar may be ascertained by titration with standard caustic alkali, litmus-paper or phenolphthalein being used as an indicator. Other methods are described on page 490.

Wine-vinegar differs in colour according as its origin is white or red wine, that derived from the former being most esteemed. It contains from 6 to 12% of absolute acetic acid, has a low sp. gr. (1.014 to 1.022), and an extract ranging from 1.7 to 2.4% (average 2.05). If the "extract" or residue left on evaporation is treated with alcohol, nearly everything dissolves except a granular residue of crude tartar, while vinegars made from malt or sugar leave a more or less glutinous residue, only sparingly soluble in alcohol. The amount of "tartar" (potassium hydrogen tartrate) contained in wine vinegar averages 0.25%. Its presence is peculiar to wine-vinegar. The tartar may be proved to be such by pouring off the alcohol and dissolving the residue in a small quantity of hot water. On cooling the aqueous solution and stirring the sides of the vessel with a glass rod, potassium hydrogen tartrate will be deposited in streaks in the track of the rod. An addition of an equal bulk of alcohol makes the reaction more delicate. Tartaric acid is occasionally added to vinegar as an adulterant, in which case the residue left on evaporation at a steam heat is viscous and highly acid. By treatment with proof-spirit any free tartaric

acid is dissolved, and may be detected in the solution by adding a solution of potassium acetate in proof spirit and stirring with a glass rod. In presence of tartaric acid, streaks and probably a distinct precipitate of potassium hydrogen tartrate will be produced. By titrating the precipitate with standard alkali, the amount of free tartaric acid in the vinegar can be determined.

Cider-vinegar is yellowish, has an odour of apples, a sp. gr. of 1.013 to 1.115, and contains 3 1/2 to 6% of acetic acid. On evaporation to dryness it yields from 1.5 to 1.8% of a mucilaginous extract, smelling and tasting of baked apples, and containing malic but no tartaric acid. Cider-vinegar usually gives slight precipitates with barium chloride, silver nitrate and ammonium oxalate, and always with lead acetate. Perry-vinegar presents similar characters.

The frequent imitation of cider-vinegar by a mixture of acetic acid and water with addition of colouring matter (generally caramel) has led to much investigation as to the means of detecting the fraud. Among the more important contributions to this subject are papers by Allen and Moor (*Analyst*, 1893, **18,** 240), G. S. Cox (*Analyst*, 1894, **19,** 89), and A. W. Smith (*J. Ame. Chem. Soc.*, 1898, **20,** 3). Cox gives the analytic results on 20 samples of cider-vinegar and 4 samples of unfermented cider. The acidity of the vinegar ranged from 2.28% to 8.4%, the solids from 1.34% to 4.0%, the ash from 0.25 to 0.52. By recalculating these results by Hehner's rule it is found that the proportion of original solids of the juice ranged from 5.51% to 16.00% and the ash from 1.94% to 4.88%.

The distinction between unadulterated cider-vinegar and the imitation made by adding colouring matter to dilute acetic acid can be easily made. The latter preparation leaves but little solid residue, almost no ash, and has but little flavour.

A. W. Smith finds that the ash of cider-vinegar differs from that of most other vinegars in the following important points:

It commences to melt and volatilise at a comparatively low temperature and gives to flame the potassium tint unobscured by that of sodium. It is low in chlorides and sulphates and high in carbonates and phosphates; about 2/3 of the phosphates are soluble in water. In the ash of other vinegars a much lower proportion of phosphates is soluble in water. The dilution of vinegar by natural water will be apt to reduce the soluble matter by the formation of calcium and magnesium phosphate.

Beer- and malt-vinegars have a high sp. gr. (1.021 to 1.025) and yield 5 to 6% of extract, containing a notable proportion of phosphates. The acetic acid varies from 3 to 6%. Barium chloride and silver nitrate frequently give considerable precipitates, owing to the presence of sulphates and chlorides in the water used in the manufacture. Some manufacturers color spirit vinegar (see above) by soaking dark malt in it and designate the product as "malt-vinegar.

Glucose- or sugar-vinegar is now extensively prepared from amylaceous materials by conversion with dilute acid, followed by fermentation and acetification. Glucose-vinegar usually contains dextrose, dextrin and, very often, calcium sulphate (see page 378). Hence it reduces Fehling's solution and usually gives abundant precipitates with barium chloride and ammonium oxalate, and frequently with silver nitrate also. When mixed with 3 or 4 times its volume of strong alcohol, glucose-vinegar gives a precipitate of *dextrin*. It is best to concentrate the sample before applying this test. Dextrose is best detected and estimated by evaporating 50 c.c. of the sample to a syrup and adding alcohol. The liquid is filtered, decolourised by boiling with animal charcoal, again filtered, the alcohol boiled off and the dextrose estimated by Fehling's solution.

Vinegar may be made by diluting acetic acid to suitable strength, colouring with burnt sugar, and flavouring with a little acetic ether. Such a product differs from malt-vinegar by containing no phosphates, and from wine- and cider-vinegars in the absence of tartaric acid and malic acid, respectively.

Hehner regards the presence of aldehyde and alcohol, causing an abundant iodoform reaction in the distillate from the neutralised sample, as evidence of fermentation, and that the sample is true vinegar. Vinegar made from sugar contains hardly any proteids, while that from malt contains about 0.7%. Vinegar prepared by acid inversion of starches usually contains a high ash with sulphates. The ash of cane-sugar vinegar is readily fusible; that of a malt or a glucose vinegar does not readily fuse. Sugar-vinegar yields an ash composed mainly of potassium salts, as raw cane-sugar is employed, not refined sugar. The estimation of potassium with a view to prove the presence of grain vinegar is useless, since both grain and raw sugar contain much potassium.

Alcohol always exists in a well-made fermentation vinegar, for manufacturers stop the process before the acetification is complete.

Vinegar may diminish in strength to the extent of fully 1% of acid in 6 months. If the alcohol is all destroyed the change is likely to be much more rapid. Vinegar should contain alcohol not only for keeping purposes, but to insure a gradual formation of acetic ether, just the same as in wine after keeping. Fermentation vinegar might be distinguished in that way, but it is easy to add alcohol to imitate a fermentation vinegar. Some manufacturers add acetic ether. There is a considerable amount of solid extract in fermentation vinegar, but in a mixture containing pyroligneous acid the quantity is much less. The solid matter differs much according to the perfection of the fermentation, and affords an indication of some value, though not so great as the amount of ash, which does not change to a great extent through the fermentation. The proportion of sulphates will afford some information as to the probable use of glucose. The estimation of total nitrogen is a valuable criterion. Grain vinegars contain a notable amount of nitrogen, although the manufacturers attempt to remove nitrogenous matters. In estimating the total nitrogen by the Kjeldahl method, the vinegar is evaporated to dryness, or at any rate to a syrup, before adding the sulphuric acid. 25 c.c. of vinegar is a convenient quantity to employ. The nitrogen found can then be calculated to its equivalent of proteins by the usual factor; but probably much of the organic nitrogen of vinegar exists in other forms. In one case Allen found 10% of the nitrogen as ammonium salts. The proportions of all constituents will differ with the strength of the vinegar. A wort which originally contained 12% of sugar and other solids will contain more nitrogen, ash and phosphates than a vinegar which originally contained only 7% of sugar. Therefore, it is desirable to adopt Hehner's plan of calculating the various constituents upon the original solids of the vinegar; 60 parts of acetic acid are theoretically produced from 90 of glucose, and hence, if the acetic acid found be multiplied by 1.5, we obtain the amount of sugar from which that acetic acid was derived. Adding to the figure thus obtained the total extractive matters still contained in the vinegar, we obtain a number representing the "original solids" of the wort. Thus, if a vinegar contain 5.2% of acetic acid and 2.8 of extract, the original solids will be 7.8+2.8=10.6. If the vinegar itself contained 0.08 of nitrogen, the original solids will contain—

$$\frac{0.08 \times 100}{10.6} = 0.75\%$$

SYNOPSIS OF RESULTS OF EXAMINATION OF TYPICAL SAMPLES OF VINEGAR.

Figures are grms. per 100 c.c.

Sample Mark	A	B	C	D	E	F	G	H	I	J	K	L	M
Specific gravity	1.0205	1.0170	1.0228	1.0160	—	1.0130	1.0185	1.0190	1.0160	1.0104	—	1.0070	1.0104
Acetic acid	6.61	6.39	5.26	4.86	4.23	5.22	5.82	5.58	5.70	3.51	4.92	4.70	7.00
Total solids	2.81	2.67	3.96	2.31	2.70	1.56	2.45	2.98	2.09	1.52	1.76	0.21	0.10
Ash	0.55	0.34	0.40	0.47	0.34	0.30	0.39	0.30	0.43	0.27	0.278	0.04	0.015
Containing: Alkalinity (K$_2$O)	0.102	0.091	0.118	—	0.024	0.03	—	0.013	—	0.086	—	trace	trace
Phosphoric acid	0.066	0.077	0.093	0.057	0.105	0.064	0.041	0.017	0.024	0.010	0.016	0.009	none
Nitrogen	0.120	0.099	0.095	0.099	—	0.052	0.097	0.104	0.062	0.014	0.016	—	0.002
Proteins	0.756	0.624	0.598	0.624	—	0.328	0.611	0.655	0.390	0.088	0.103	—	0.013
"Original solids"	12.73	12.26	11.85	9.60	9.35	9.39	11.18	11.35	10.64	6.81	10.02	7.26	10.60
Per 100 parts of original solids: Ash	4.32	2.78	3.37	4.92	3.64	3.20	3.49	2.64	4.04	3.94	2.77	0.55	0.14
Phosphoric acid	0.52	0.63	0.79	0.60	1.16	0.68	0.37	0.15	0.225	0.14	0.16	0.120	none
Nitrogen	0.95	0.816	0.80	1.03	—	0.56	0.87	0.93	0.582	0.206	0.16	—	0.019
=Proteins	5.98	5.14	5.04	6.49	—	3.53	5.48	5.86	3.670	1.30	1.03	—	0.120

B, C, D, and probably A appear to be from mixtures of malted and unmalted grain, the starch entirely hydrolysed by diastase.

E is the average of the first seven samples reported by Hehner.

F and G are from mixtures of malted and unmalted grain with addition of sugar.

H and I are chiefly from rice hydrolysed by sulphuric acid.

J and K were made from sugar; J contained possibly a little malt.

L was reported by Dr. Hill as containing between 70 and 80 per cent. of wood acid.

M is a very pale vinegar made by mixing distilled vinegar with a little of the same sample undistilled.

It possesses an appetising taste and smell.

In this manner one can eliminate the differences caused by irregularity in the strength of various samples of vinegar and reduce the results to a kind of common denominator. As a matter of fact, the loss of acetic acid in the process of manufacture averages some 30%, so that the proportion of original solids calculated in the above manner is always below the truth. Hence a nearer approximation to accuracy would be obtained by multiplying the acetic acid by 2.25, instead of 1.5, before adding the extract, but the change would involve confusion, and it is best to adhere to the mode of calculation originally suggested by Hehner.

Wood vinegar is a name sometimes applied to pyroligneous acid.

Aromatic vinegar is a product obtained by distilling a metallic acetate, usually crystallised cupric acetate. The presence of acetone and other bodies imparts an agreeable aroma. A small addition of camphor or essential oil is often made.

Mineral Acids in Vinegar.—Very weak vinegar is liable to a putrid fermentation, to prevent which the addition of 1 gallon of sulphuric acid to 1000 gallons of vinegar (about 0.185%) was permitted by a British Excise regulation. This addition is now known to be unnecessary with good vinegar and is abandoned by the best makers, though the practice is not obsolete, and the legal proportion of sulphuric acid has been occasionally largely exceeded. In addition to sulphuric acid, hydrochloric acid has been occasionally added to vinegar, but the adulteration of vinegar with mineral acids is now very rarely practised.

For detecting mineral acids in vinegar several tests have been devised, but the most are either untrustworthy or deficient in delicacy. Some are applicable to the detection of sulphuric acid only, whilst others include hydrochloric and other mineral acids also. The employment of barium chloride and silver nitrate for the detection of sulphuric and hydrochloric acids, respectively, has led several analysts into error, owing to the presence naturally of sulphates and chlorides in the water employed in the manufacture of the vinegar.

Another circumstance which complicates the problem is that the addition of a mineral acid in moderate quantity merely decomposes the acetates naturally present in the vinegar, with liberation of acetic acid and formation of sulphates or chlorides. Hence, only the acid beyond that required for the decomposition of the acetates can exist in the free state, and to the presence of such free mineral acids only

can objection reasonably be taken, unless the mineral acid used is contaminated with arsenic.

Acetates and most other salts of organic acids decompose by ignition into carbonates, having an alkaline reaction to litmus, while sulphates and chlorides of the lighter metals are unchanged on ignition and possess a neutral reaction. Hence, if the ash of a vinegar has a sensibly alkaline reaction, acetates must have been present in the original vinegar and no free sulphuric or hydrochloric acid. To determine the amount of free mineral acid it is sufficient to neutralise the vinegar with standard sodium hydroxide before evaporation to dryness (the same process serves for a determination of the total free acid), ignite the residue, and titrate the aqueous solution of the ash with standard acid. If the free acid originally present was wholly organic, the ash will contain an equivalent amount of alkaline carbonate, which will require an amount of standard acid for its neutralisation exactly equivalent to the amount of standard alkali originally added to the vinegar. Any deficiency in the amount of standard acid required for neutralisation is due to the *free mineral acid* originally present in the vinegar. More accurate results are obtained if the amount of standard alkali added before evaporation is insufficient for the complete saturation of the acetic acid, but more than enough for the neutralisation of all mineral and fixed organic acids which may be present. By thus proceeding, decinormal alkali and acid may be employed (50 c.c. of the vinegar being used), and thus sharper readings obtained.

The *total chlorine*, existing as chlorides, cannot be ascertained in vinegar by direct precipitation with silver nitrate. For a correct assay, 50 c.c. of the vinegar should be neutralised with alkali, evaporated to dryness, the residue ignited, dissolved in water, and the aqueous solution precipitated with excess of calcium sulphate or nitrate to remove phosphates. The filtrate from this precipitate may be precipitated by, or titrated with a solution of silver nitrate.

The sulphuric acid and sulphates may be precipitated by the direct addition of barium chloride to the diluted vinegar, but the figure has little value.

Free sulphuric acid, as distinguished from sulphates, may be estimated with considerable accuracy by evaporating 100 c.c. of the vinegar to a small bulk and then adding to the cold concentrated liquid 4 or 5 times its volume of alcohol. Sulphates are precipitated, while free sulphuric acid remains in solution. The filtered liquid is diluted,

the alcohol boiled off and the sulphuric acid precipitated with barium chloride. The precipitate is filtered off, washed, dried, ignited and weighed. Its weight, multiplied by 0.4206, gives the weight of sulphuric acid in the quantity of vinegar taken. In a vinegar free from chlorides this process gives results in accordance with Hehner's process, but in their presence the mineral acid found is deficient by the amount of sulphuric acid required to decompose the chlorides. This difficulty may be obviated by treating the vinegar with excess of silver sulphate solution before evaporation, by which treatment any free hydrochloric is also estimated as sulphuric acid.

An ingenious method of detecting *free sulphuric acid* in vinegar and wine has been described by Casali. 20 grm. weight of the sample is ground up in a mortar with about 80 grm. of finely powdered porcelain (previously treated with hydrochloric acid to remove every trace of free alkali), so that the mixture is not moist to the touch. The whole is then ground up with 50 c.c. of ether (previously agitated with magnesia and water to neutralise any trace of acid), filtered and washed with ether. The filtrate is then shaken with a little distilled water, the ether distilled off and the residue precipitated with barium chloride; 0.0005 grm. of free sulphuric acid can be readily detected by this method.

A very simple, and apparently reliable method of detecting free *mineral acids* in vinegar has been described by A. Ashby. A solution of logwood is prepared by pouring 100 c.c. of boiling water on about 2 grm. of fresh logwood chips, and then allowing the decoction to stand for a few hours. Separate drops of this solution are spotted on the surface of a flat porcelain dish or on the cover of a porcelain crucible, and evaporated to dryness over a beaker of boiling water. To each spot a drop of the suspected sample (previously concentrated, if thought desirable) should be added, and the heating continued till the liquid has evaporated. If the vinegar is pure the residue will be found to have a bright yellow colour, but in presence of a very small proportion of mineral acid the residue assumes a red colour.

If the proportion of mineral acid is very small, the red colour is destroyed on adding water to the residue, but is restored on evaporating, except in the case of nitric acid.

Tartaric acid in vinegar may be detected as described under Wine-vinegar, of which it is a normal constituent.

Oxalic acid may be detected by evaporating 20 c.c. of the vinegar

to a small bulk, diluting the residue with water, and adding calcium-acetate solution or a mixture of ammonium acetate and calcium chloride. Any oxalic acid causes the formation of white calcium oxalate.

Arsenic has been occasionally met with in vinegar, and may be introduced by the addition of impure hydrochloric or sulphuric acid. It may readily be detected by Marsh's or Reinsch's test.

Lead and copper may be detected as described on pages 63 and 569.

Zinc is occasionally present in vinegar. It may be detected by boiling down the vinegar to dryness with nitric acid, dissolving the residue in acidulated water, passing hydrogen sulphide, filtering from any precipitate and then adding ammonium acetate, when white zinc sulphide will be thrown down if the metal is present. A less satisfactory method is to neutralise the greater part of the free acid in the original vinegar by ammonia, and then at once passing in hydrogen sulphide.

Cayenne pepper and ginger, are sometimes added to vinegar to confer pungency. They may be detected by neutralising the concentrated vinegar with sodium carbonate and *tasting* the liquid.

Flies and so-called "eels" are often found in vinegar. They are readily detected by the microscope, and may be destroyed by raising the temperature of the liquid to 100°.

Analysis of Commercial Vinegar.—The following is a summary of some of the processes provisionally suggested by the *Association of Official Agricultural Chemists*, and published by the *United States Bureau of Chemistry*, Washington, D. C. (*Bulletin* 107, United States Department of Agriculture).

Microscopic examination should be made of the sediment, but the sample for chemical examination should be filtered and tested as soon as possible. All results are expressed by weight, but c.c. are regarded as equivalent to grm. in routine testing. The sp. gr. is determined in the usual way.

Alcohol.—100 c.c. are exactly neutralized with sodium hydroxide and 40 c.c. distilled. The distillate is redistilled until 20 c.c. are collected. This distillate is cooled to 15.5°, made up to 20 c.c. and the alcohol ascertained in the usual way.

Total Solids.—10 c.c. are evaporated to syrupy consistence, then dried in an oven for 2 1/2 hours, cooled and weighed.

Ash.—This is determined in the usual way from the above residue.

Alkalinity and Solubility of Ash.—25 c.c. of the sample are

ashed, extracted repeatedly with hot water, collecting the undissolved material by passing the water through an ashless filter. The water solution is titrated with N/10 acid and methyl-orange. The filter is dried, burned and the residue weighed.

Phosphoric Acid.—This is ascertained in both the water-soluble and insoluble portions of the ash by the standard methods of fertiliser analysis.

Total Acidity.—A suitable amount diluted until it has no interfering colour is titrated with N/10 alkali using phenolphthalein as indicator. One c.c. of the alkali is equivalent to 0.0060 acetic acid.

Volatile Acids.—15 c.c. are heated to boiling in a flask, using a little tannin if necessary to prevent foaming. The flame is then lowered and a current of steam passed through, and continued until 15 c.c. of the liquid in the condenser show no acidity with sensitive litmus-paper. The combined distillate is titrated for total volatile acids.

Fixed Acids.—The figure for volatile acids is deducted from that for total acids; the remainder multiplied by 0.817 will give sulphuric acid, or, by 1.117, malic acid. If tannin has not been added, the undistilled portion may be directly titrated with N/10 acid. One c.c. of this is equivalent to 0.0049 sulphuric acid or 0.0067 malic acid.

Potassium Hydrogen Tartrate.—Use the method on page 545.

Tartaric Acid.—Treat an alcoholic solution of the residue, obtained as directed in the immediately preceding process, with an alcoholic solution of potassium acetate and stir mixture with a glass rod, drawing the latter along the sides of the beaker. Crystals of acid potassium tartrate will separate if tartaric acid is present. Approximate quantitative results may be obtained by titrating this precipitate with standard alkali.

Mineral Acids.—For the logwood method see page 503. Another method is as follows: 5 c.c. of the sample are diluted with 10 c.c. of water, then a few drops of a solution of methyl-violet (1 part of colour to 10,000 of water) are added. If the liquid becomes blue or green, mineral acid is present.

The amount of mineral acid ascertained is by Hilger's method: 20 c.c. are exactly neutralised with N/2 alkali, using sensitive (neutral) litmus-paper as the indicator. The liquid is evaporated to 1/10 its volume, a few drops of the dilute solution of methyl-violet added (see above) and, if the liquid is not clear, a few c.c. of water added, then titrated with N/2 sulphuric acid until the solution becomes green or

blue. The difference, in c.c., between the N/2 alkali required and the N/2 acid required multiplied by 0.1225 expresses the mineral acid present in terms of sulphuric acid.

Acetates.—Many of these are extensively used in the arts and medicine. Their analytical characters and the general methods adopted for their assay have been, in great measure, already described. The following observations, therefore, have reference chiefly to the detection of impurities and adulterations in commercial acetates. Sections treating of acetic esters and acetates containing nitrogenous bases will be found in other parts of this work.

Potassium Acetate, $KC_2H_3O_2$.—This exists in some vegetable secretions. It is deliquescent, very soluble in water and alcohol and neutral to litmus. It fuses at incipient redness, and at a higher temperature decomposes, leaving potassium carbonate. The amount of acetate present in commercial samples may be ascertained by the general methods given on page 509.

Commercial potassium acetate is liable to contain sulphates, chlorides and carbonates; iron, lead, copper and zinc; arsenic is occasionally present. It is sometimes intentionally adulterated, calcium acetate and potassium sulphate, potassium tartrate or potassium carbonate being employed.

Potassium acetate being soluble in alcohol, any admixture of *sulphates, tartrates* or *carbonates* may be detected and estimated by treatment with that solvent. *Carbonate* is indicated more precisely by alkaline reaction; precipitation by chloride of calcium; power of decolorising iodised starch; and effervescence on adding an acid.

Sodium acetate, $NaC_2H_3O_2$, closely resembles the potassium salt, but crystallises with 3 molecules of water. It is liable to contain the same foreign matters as potassium acetate. Crude sodium acetate often contains tarry matters derived from the pyroligneous acid employed in its preparation. Its supersaturated solution has been used for filling foot-warmers.

Ammonium Acetate, $(NH_4)C_2H_3O_2$.—This salt is generally met with in solution, but may be obtained in the solid state, when it is apt to contain acetamide.

Ammonium acetate is liable to contain much the same impurities as the potassium salt, and may be examined in a similar manner. It should be wholly volatile on ignition.

Calcium Acetate, $Ca(C_2H_3O_3)_2$.—This crystallises with diffi-culty in prismatic needles containing 1 molecule of water. It is de-composed by heat into acetone, and calcium carbonate.

Calcium acetate should be completely soluble in water and in proof spirit. An insoluble residue may consist of calcium sulphate or carbon-ate. The solution should give no precipitate with silver nitrate or barium chloride. Potassium ferrocyanide colours the solution blue if the sample contains iron, and brown if copper is present.

Assay of "Acetate of Lime."—This is the commercial name for calcium acetate obtained from crude pyroligneous acid. It is often very impure, containing tarry matter; calcium hydroxide, carbonate and sulphate and salts of the homologues of acetic acid. Its assay is of importance and somewhat difficult. If the salt is ignited, and the amount of acetic acid calculated from the weight of the residual calcium carbonate or from the amount of normal acid the residue will neutralise, very erroneous results may be obtained.

Calcium formate has been found in crude acetate, the proportion sometimes reaching 4 or 5%. When operating on the large scale, the presence of formates is unmistakable. On crystallising out sodium acetate as completely as possible, a dense syrupy liquid is left which contains sodium formate, reduces silver and mercuric salts, and evolves carbon monoxide when treated with excess of sulphuric acid.

A method of assay, much used in the neighborhood of Manchester, England, has been described by H. Grimshaw: 10 grm. of the sample of crude acetate are dissolved in boiling water, and 20 grm. of crystallised sodium sulphate added. The liquid is raised to boil-ing, cooled, diluted to 250 c.c., and allowed to stand for 12 hours. The calcium will then have separated as crystalline calcium sulphate. The liquid is filtered, the precipitate washed with hot water, and the filtrate made up to 500 c.c. 50 c.c. of this solution ($=1$ grm. of the sample) should then be evaporated to dryness at $100°$, and somewhat further dried in an air-bath. The residue is ignited at a red heat over a good bunsen burner for half an hour, allowed to cool, and treated with 10 c.c. of $N/1$ hydrochloric acid, using a cover to avoid loss. The solution is boiled well to drive off carbon dioxide, filtered, the residual carbon washed, and the filtrate titrated with decinormal sodium hydroxide, using methyl-orange or litmus as an indicator. Each c.c. of normal acid found to have been neutralised by the ash represents 0.060 grm. of acetic acid ($C_2H_4O_2$), or 0.079 grm. of calcium

acetate, in the liquid (= 1 grm. of the sample) evaporated. Great care is requisite in conducting the titration, as a very small difference in the volume of alkali required makes a sensible change in the result. The portion of the sample taken for the analysis should be finely powdered, and if the solution in water be appreciably acid it should be cautiously neutralised with sodium hydroxide before adding the sodium sulphate. Grimshaw found this process to give results ranging from close agreement to about 2% in excess of those obtained from the same samples by distillation with phosphoric acid. The results are not vitiated by the presence of calcium carbonate or other insoluble calcium compounds in the sample.

Allen found a tendency to incomplete decomposition of the acetate if too low a temperature is employed. He suggested to evaporate a measure of solution representing 5 grm. of the sample, and ignite at a moderate red heat in a muffle, subsequently moistening the ash with hydrogen peroxide to oxidise any sulphides which may have been formed.

The distillation process given in the preceding edition of this work was communicated by Stillwell and Gladding, being an improvement on the process published previously by them. A further communication by Stillwell will be found in *J. Soc. Chem. Ind.*, 1904, 23, 305. The following process, essentially the same, is given by H. C. Sherman (Methods of Organic Analysis) as now in general use. For an extended account of methods of assaying commercial calcium acetate, see a paper by Grosvenor in *J. Soc. Chem. Ind.*, 1904, 23, 530.

A 300 c.c. flask is fixed at an angle of about 60° from the perpendicular and connected with a nearly vertical condenser while another tube passing through the stopper of the flask provides for the introduction of water, drop by drop, during distillation. The flow of water should be under complete control. The weighed material (2 grm.) finely ground is transferred to the flask, 15 c.c. of 50% phosphoric acid and 25 c.c. of water added, taking care that the water washes down any powder or acid that is in the neck of the flask. The apparatus is connected and the distillate collected in a receiver containing 50 c.c. of water. During the process, the volume of the liquid should be kept at 40 c.c. as near as may be, by admitting water free from carbonic acid, adding it so that the drops fall on the side of the flask and not directly into the distilling liquid. It is stated that it is advantageous to use water containing a little phosphoric acid. The

operation is continued until the distillate is no longer acid, which usually requires about ninety minutes. The distillate is titrated with fresh standard alkali.

This modification of the usual methods avoids the danger of phosphoric acid being carried over mechanically. In grinding the sample care must be taken not to lose moisture. It is recommended to evaporate the titrated distillate and apply the usual qualitative test for phosphoric acid to make sure that no appreciable amount of this has been carried over. The distillate, of course, contains all the other volatile acids present of which the salts are present in the sample.

The phosphoric acid employed for the distillation must be free from nitric acid, which if present may be eliminated by adding a little ammonia, and heating the acid to fusion in platinum. If either the phosphoric acid or the sample itself contains chlorides, some silver sulphate must be added to the contents of the retort. Oxalic acid may be substituted for the phosphoric acid, the solution being filtered from the precipitated calcium oxalate before introduction into the retort. Hydrochloric acid may be used instead, provided that the amount which passes into the distillate be estimated and subtracted from the total acidity as deduced from the titration. Sulphuric acid should not be used, as its reaction on the tarry matters occasions the formation of sulphurous acid, which increases the acidity of the distillate.

FIG. 86.

Gladding has recently (*J. Indust. and Eng. Chem.*, 1909, 1, 250) reported the latest modification of the process as carried out in his laboratory with satisfaction. The apparatus shown in Fig. 86 is used. 2 grm. of the sample and 30 c.c. of water are introduced into A (about 300 c.c. capacity); 10 c.c. of phosphoric acid (sp. gr. 1.7) are added and the liquid boiled gently for about 90 minutes while the volume is kept at 50 c.c. The distillate, condensed in C, is received in B which contains 30 c.c. of standard alkali. At the end of 90 minutes' distillation the contents of B are titrated. The distillation should be continued until the distillate is neutral. Phenolphthalein is used as an indicator. A
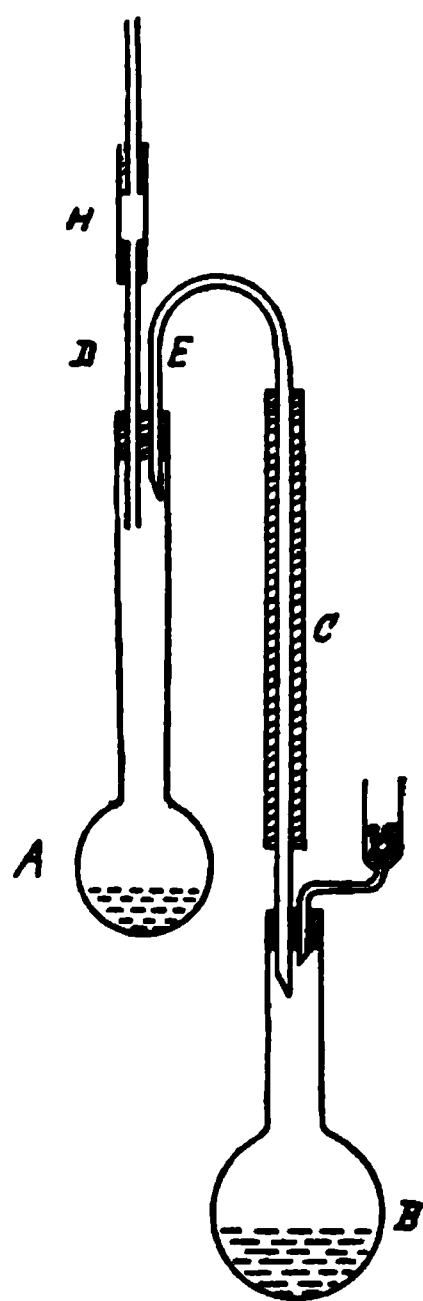
blank experiment should be made. The volume of liquid in A is kept at 50 c.c. by adding water drop by drop through the tube H. Gladding refers to a paper by Fresenius and Grünhut (*Zeit. anal. Chem.*, 1908, 597) in which these authors disapprove of his method, but he shows that they did not subject it to proper comparison and that the method is trustworthy.

When *pure* calcium acetate is assayed by either of the foregoing methods accurate results may be obtained, but when commercial samples are examined the errors may sometimes become serious. On the whole, the method of distillation with phosphoric acid is the most accurate, but, unless carefully performed, the results are liable to be below the truth, from incomplete volatilisation of the acetic acid, while on the other hand, they may be excessive if nitric or other volatile acid be present in the phosphoric acid used.[1]

Magnesium Acetate.—Basic magnesium acetate has been recommended as an antiseptic.

Aluminum Acetate.—This salt is employed in solution by calico-printers under the name of "red-liquor." It is usually prepared by precipitating a solution of alum or aluminum sulphate by means of calcium or lead acetate, and filtering or syphoning off from the precipitated calcium or lead sulphate. When prepared by means of alum, the product necessarily contains potassium sulphate or ammonium sulphate (according to the kind of alum used), and, as an excess of the precipitant should be avoided, aluminum sulphate is always to be expected. Owing to calcium sulphate being somewhat soluble in water, it will be met with in red-liquors prepared with calcium acetate. Such red-liquor is inferior to that prepared by lead acetate. Good red-liquor contains the equivalent of from 3 to 5% of

[1] The following results, reported by Allen from the same sample of "acetate of lime" by different methods, show the nature and direction of the errors to which the various processes are liable:

	Acetic Acid. Per cent.
By distillation with phosphoric acid and titration of distillate,	47.4
By distillation with phosphoric acid and titration of distillate,	48.0
By distillation with sulphuric acid and titration of distillate,	48.6
By distillation with oxalic acid and titration of distillate,	48.3
By distillation with oxalic acid and titration of distillate,	48.4
By Fresenius' method,	53.4
By Fresenius' method,	53.2
By ignition and weighing the calcium carbonate,	53.2
By ignition and titration of residue,	53.2
By ignition and titration of residue,	53.8
By ignition and titration of residue,	54.0
By boiling with sodium carbonate, and titrating filtrate,	56.4
By boiling with sodium carbonate, and titrating filtrate,	56.4
By boiling with sodium carbonate, and titrating filtrate,	57.6

Improvements in the manufacture of calcium acetate render the discrepancies resulting from the employment of different methods of assay less striking than formerly.

alumina, and twice that proportion of acetic acid, and has a gravity of 1.120, but it is sometimes met with as low as 1.087. Sodium carbonate is often added to red-liquor to neutralise excess of acid.

Iron Acetates.—Both ferrous and ferric acetates are employed in the arts. A crude variety of iron acetate is extensively manufactured by dissolving iron in pyroligneous acid.

Pyrolignite of Iron, Iron Liquor or Black Liquor.--For use by calico-printers, a liquid consisting chiefly of a solution of ferrous acetate, but always containing more or less ferric acetate, is prepared by acting on scrap-iron by crude pyroligneous acid of 1.035 to 1.040 sp. gr. A purified acid gives less satisfactory results. The product, which is a deep black liquid, has a gravity of 1.085 to 1.090, and is concentrated by boiling till it is about 1.120, when it contains about 10% of iron. It is then ready for use, and is known as "printers' iron liquor." Much iron liquor is now made as high as 1.140. For use by dyers, the liquid is not concentrated by evaporation, but the gravity is raised by the addition of ferrous sulphate (copperas), by which a more suitable product is said to be obtained than is yielded by iron acetate alone. As a 5% solution of crystallised ferrous sulphate has a gravity of 1.026, the addition of 1/2 pound of copperas to the gallon of "black liquor" will raise it from 1.085 to 1.111. As much as 124 grm. of ferrous sulphate per 1000 c.c. has been met with in iron liquor. The sulphate may also result from the addition of sulphuric acid to the pyroligneous acid employed for dissolving the scrap-iron. Iron sulphate may be detected and estimated by precipitating the diluted black liquor with barium chloride. 233 parts of the precipitate represent 278 parts of crystallised ferrous sulphate. Black liquor is frequently adulterated with common salt, a 5% solution of which has a gravity of 1.036. It may be detected and estimated by adding nitric acid and precipitating the diluted liquor with silver nitrate. Iron chlorides may also be present owing to the addition of hydrochloric to the pyroligneous acid. Hence the chlorine must not be assumed to exist as common salt without further examination. This is best effected by heating the liquid with nitric acid, adding barium nitrate to separate the sulphates, precipitating the iron and excess of barium by ammonium hydroxide and carbonate, evaporating the filtrate to dryness, and igniting the residue, when any common salt will remain. *Tannin* is stated to be occasionally added to iron liquor.

Ferrous acetate is sometimes made by decomposing a solution of

ferrous sulphate by calcium acetate. The liquor has usually a gravity of 1.11, and contains calcium sulphate.

Ferric acetate is sometimes preferred by dyers and printers to the ferrous salt. It is occasionally prepared by decomposing iron-alum or ferric sulphate by lead acetate. The product must be free from excess of the lead salt, and, for some purposes, excess of ferric sulphate must be avoided.

Tincture of ferric acetate may be prepared by mixing alcoholic solutions of potassium acetate and ferric sulphate, and filtering from the precipitated potassium sulphate.

Lead Acetates.—These include neutral acetate, Pb $(C_2H_3O_2)_2$, often called "sugar of lead," and several basic- or oxyacetates, all of which are more or less soluble in water, the solutions possessing an alkaline reaction and giving a precipitate of lead carbonate by the action of carbon dioxide. A solution of neutral lead acetate is but little affected by carbon dioxide. By suspending basic acetate in water and passing carbon dioxide through the liquid as long as it has an alkaline reaction, the lead is separated as an insoluble carbonate, and may be filtered off, washed, ignited in porcelain (apart from the filter) till bright yellow when cold, and weighed as lead monoxide. The lead remaining in permanent solution exists as *acetate*, and may be ascertained by precipitation as sulphate or chromate.

A better and simpler method for detecting basic acetate in a sample is to dissolve it in recently-boiled water, filter, and then add to the clear solution an equal measure of a 1% solution of mercuric chloride. A white precipitate proves the presence of basic acetate. The assay may also be conducted by methods given on page 509.

Fresenius recommends the following indirect method for the assay of pyrolignite and lead acetate: 10 grm. of the sample are dissolved in water in a flask holding 500 c.c., 60 c.c. of normal sulphuric acid are added, and the water up to the mark. An extra 1.3 c.c. of water is added to compensate for the bulk of the precipitated lead sulphate. The flask is closed, well shaken, and the liquid allowed to settle. 100 c.c. of the clear liquid are taken out, precipitated with barium chloride, and the precipitate collected, washed, ignited and weighed. Its weight, multiplied by 0.4206, is subtracted from 0.588 grm. (the weight of acid added to each 100 c.c. of the liquid). The remainder, multiplied by 113.7 gives the percentage of lead monoxide

in the sample. Another 100 c.c. of the clear liquid are drawn off and titrated with N/1 sodium hydroxide, using litmus as an indicator. Multiply the number of cubic centimetres of alkali used by 0.060, subtract from this the previously obtained weight of barium sulphate multiplied by 0.515 (= the free sulphuric acid expressed in terms of acetic acid), and the remainder, multiplied by 50, will be the percentage of acetic acid in the sample.

Basic Lead Acetate.—Solutions of basic lead acetate have been long used in medicine and formulas for their preparation are given in pharmacopœias. A solid basic acetate and a solution are much used as clarifying agents in saccharimetry. For the method of preparing such a solution see page 308.

Cupric Acetates.—Several of these salts are known and extensively used in the arts. They are prepared by the action of acetic acid on copper oxide or carbonate or upon metallic copper with access of air. The neutral acetate is freely soluble in water, but several basic acetates exist. They are of different shades of color, and are known as blue and green verdigris.

Verdigris of good quality is dry, soluble in dilute acetic acid, sulphuric acid and ammonium hydroxide. It should not contain more than 4% of impurities. A good sample will correspond to about the following composition: cupric oxide, 43.5; acetic anhydride, 29.3; water, 25.2; and impurities, 2.0. It is frequently adulterated. Sand, clay, pumice and chalk; barium, calcium and copper sulphates; and iron and zinc compounds, are sometimes present. Zinc in verdigris is usually due to the use of sheets of brass instead of copper for corrosion by acetic acid.

On dissolving the sample in dilute hydrochloric acid, any *sand*, *clay*, *pumice*, or *barium sulphate* will be left insoluble, and may be collected and weighed. (About 3% of insoluble matter is allowable in verdigris. If the residue amounts to 6% the sample is inferior. *Calcium sulphate* in large proportion may be left partially in the insoluble residue). If the sample effervesced on addition of acid, *a carbonate* is present, though it may be that of copper. From a measured portion of the solution in acid the *sulphates* may be precipitated by barium chloride, the precipitate collected and weighed.

For the detection of the *metals*, the sample should be ignited, the residue dissolved in hydrochloric acid, and the copper precipitated from the diluted liquid by a current of hydrogen sulphide. In the filtrate

the excess of hydrogen sulphide is destroyed by bromine water, the liquid nearly neutralised by ammonium acetate, and then boiled with ammonium acetate. The precipitate, when washed and ignited, is ferric oxide. The filtrate from the iron precipitate is treated with hydrogen sulphide and any white zinc sulphide filtered off, carefully roasted and weighed as oxide. From the filtrate, the calcium is precipitated by ammonium oxalate. The precipitate yields calcium carbonate on gentle ignition, the weight being equal to the chalk in the quantity of the sample taken. The calcium may be determined more readily, but less accurately, by dissolving the sample in hydrochloric acid, precipitating the iron by bromine and ammonium hydroxide, and then at once treating the filtrate with ammonium oxalate. Of course, it does not follow that all the calcium found exists as chalk, unless sulphates are absent.

HOMOLOGUES OF ACETIC ACID. Lower Fatty Acids.

Acetic acid is the most important and best known of the homologous series called "the fatty acids." These acids have the general formula $C_nH_{2n}O_2$. The lower members of the series are volatile liquids closely resembling acetic acid. The higher members of the series are insoluble in water, not volatile without decomposition, and solid at ordinary temperatures. Many fatty acids are known, but the greater number are of very limited importance.

The higher members of the fatty acid series are almost exclusively obtained by the saponification of the fixed oils, fats and waxes, and such of them as require description will be considered in the section treating of these bodies. The present article is limited to a consideration of the lower members of the series, sensibly volatile or soluble in water, and hence liable to occur under the same circumstances as acetic acid.

With the exception of the first three, all the members of the acetic series of acids are capable of isomeric modification. The number of such modifications increases rapidly with the number of carbon atoms in the molecules, and many have been obtained.

The following table gives the names of the normal and isoacids of the acetic series up to the member with 7 carbon atom. Above caproic acid the modifications have been imperfectly differentiated. A

table of the still higher members of the series will be given in the section on "Saponification."

From this table it will be observed that the b. p. of the normal fatty acids show a tolerably regular increase of 18° to 22° for each increment of CH₂ in the formula. The isoacid in each case boils at a lower temperature than the normal and has also lower sp. gr. The sp. gr. and solubility of the fatty acids, as also the solubility of many of their salts, decrease with an increase in the molecular weight. The ethers of the fatty acids similarly diminish in solubility and volatility with each increase in the number of carbon atoms.

Empiri-cal Formula	Name	Constitutional Formula	Boil-ing Point ° C.	Specific Gravity at 0° C.	Solubility in Water
CH₂O₂	Formic acid, . . .	H.COOH	100		{ M i scible in all pro-portions
C₂H₄O₂	Acetic acid,. . . .	CH₂.COOH	119		Do
C₃H₆O₂	Propionic acid, . .	CH₂.CH₂.COOH	140	1.016	Do
C₄H₈O₂	{ Normal butyric acid	CH₂.(CH₂)₂.COOH	163 .	.9817 . .	Do
	{ Iso-butyric acid; or dimethacetic acid,	CH(CH₂)₂.COOH	154	. . .9598	Soluble
C₅H₁₀O₂	Normal pentoic or valeric acid,. . .	CH₂.(CH₂)₃.COOH	185 .	.9577 . .	{ Sparingly (1 in 30)
	Iso-pentoic acid; ordinary valeric acid; or iso-prop-acetic acid, . . .	CH(CH₂)₂CH₂.COOH	. 175	. . .9536	Do
C₆H₁₂O₂	Normal caproic acid	CH₂.(CH₂)₄.COOH	205 .	.9450 . .	{ Nearly in-soluble
	Iso-caproic acid, .	CH(CH₂)₂.(CH₂)₂.COOH	. 199	. . .9310	Do
C₇H₁₄O₂	Normal œnanthylic acid, ...	CH₂.(CH₂)₅.COOH	224 .	.9345 . .	{ Almost insoluble
	Iso-œnanthylic acid	CH(CH₃)₂.(CH₂)₃.COOH	. 213	. . . .	Do

As a rule, the isoacids present very close resemblances to the corresponding normal acids, their lower gravities and b. p. and greater susceptibility to oxidation being the most marked distinctions. In some cases, differences are observable in the solubility and crystallisability of the salts.

As a class, the lower members of the acetic acid series may be separated from most other organic acids (except lactic acid) by treating the aqueous solution with finely-ground lead monoxide in quantity sufficient to render it slightly alkaline. On filtering, the lead salts of most organic acids will be left insoluble, while those of the acetic series will be found in the filtrate.

The separation of the lower acids of the acetic series from each other cannot usually be effected readily; the most satisfactory methods are based on the following principles:

The lowest members of the series are the most readily soluble in aqueous liquids, formic, acetic, propionic and normal butyric acid, being soluble in all proportions. All but formic and acetic acids are separated from their aqueous solutions by saturating the liquid with calcium chloride, when they rise in the form of oils. A more perfect separation from acetic and formic acids of the acids higher than valeric may be effected by shaking the acidulated aqueous solution with ether, which dissolves the higher homologues together with more or less of the lower. On agitating the ethereal layer with a strong solution of calcium chloride the formic and acetic acid pass into the latter, and by repeating the treatment may be perfectly removed from the ether, with little or no loss of the higher homologues.

The lower members of the series are most active. Hence, if an amount of alkali insufficient for complete neutralization be added to a solution containing the free acids, and the liquid be then distilled, the higher members of the series pass over in the free state, while the lower members remain behind as fixed salts.

If sodium hydroxide is added to a mixture of butyric and valeric acids in quantity insufficient to neutralise the whole, and the liquid be then distilled, the distillate will consist of pure valeric acid and the residue will contain mixed sodium butyrate and valerate; or else the distillate will contain the whole of the valeric acid and some butyric acid, and the residue will consist entirely of butyrate of sodium. In either case, a portion of one of the acids is obtained free from the other. In the first case, the residue of mixed valerate and butyrate may be treated with sufficient dilute sulphuric acid to neutralise half of the sodium hydroxide originally used, and the mixture redistilled, when a fresh quantity of valeric acid will be obtained, either pure or mixed with butyric acid according to the relative proportions of the two acids present in the original mixture. In the latter case, by partially neutralising the distillate with alkali, and again distilling, a further separation may be effected, and by repeating the operation in a judicious manner two or even more of these volatile fatty acids may be separated fairly well from each other.

Although the foregoing method is well suited to the separation of normal butyric and valeric acids, the principle is wholly at fault when iso-valeric acid is in question, for this acid completely decomposes normal butyrates.

An approximate separation of the homologues higher than valeric acid can be effected by a fractional crystallisation of their barium salts. The following is the order in which the barium salts are deposited:

From aqueous solutions.	From alcoholic solutions.
1. Barium caprate.	1. Barium caprylate.
2. Barium pelargonate.	2. Barium œnanthylate.
3. Barium caprylate.	3. Barium pelargonate and caprate.
4. Barium œnanthylate.	
5. Barium caproate.	4. Barium caproate.

The aqueous or alcoholic solution of the acid is neutralised with standard aqueous or alcoholic solution of sodium hydroxide (according as the crystallisation is to be effected from an aqueous or alcoholic solution), an amount of barium chloride equivalent to the alkali is next added, and the resultant liquid evaporated and allowed to deposit crystals. The crops of crystals from an aqueous solution may be washed with hot alcohol, the washings containing the salts in the reverse order of their deposition from alcoholic solution.

Another method of detecting and estimating acids of the acetic series when in admixture with each other is based on the different composition of their barium salts, the process being as follows: The free acids obtained by distillation are saturated by barium carbonate or by the cautious addition of baryta water (using phenolphthalein to indicate the point of neutrality), the latter method being preferable for the higher numbers of the series. In this way, neutral barium salts are formed, which may be obtained in the anhydrous state by evaporating off the water and drying the residue at 130°. These barium salts contain percentages of barium dependent on the atomic weights of the fatty acids present. On moistening the residue with sulphuric acid and then igniting, an amount of barium sulphate is obtained proportional to the percentage of barium contained in the salt of the fatty acid present. Instead of weighing the barium sulphate, a standard solution of baryta water may be employed and the weight of barium (or its equivalent of barium sulphate) calculated from the volume of solution employed. This method also serves as a useful check on the determination of the weight of barium sulphate. The following table shows the proportions of barium contained in, and of barium sulphate producible from the barium salts of the lower acids of the acetic series:

Name of salt	Barium, %.	Barium sulphate, %.
Barium formate............................	70.25	119.47
" acetate............................	53.73	91.37
" propionate........................	48.41	82.13
" butyrate..........................	44.05	74.91
" valerate..........................	40.41	68.73
" caproate..........................	37.33	63.48
" œnanthylate......................	34.68	58.98
" caprylate.........................	32.39	55.08
" pelargonate......................	30.38	51.66
" caprate..........................	28.60	48.64

From this table it will be seen that the pure barium salts of the lower acids of the acetic acid can very readily be distinguished from each other by estimating the percentage of barium contained in them. In the case of mixtures of two acids the identity of which is established, the proportions in which the two are present may be calculated from the following formula, in which x is the percentage of barium salt of the lower fatty acid in the mixed barium salts obtained; P, the percentage of barium sulphate yielded by the mixed barium salts on treatment with sulphuric acids; B, the percentage of the same theoretically obtainable from the pure salt of the lower fatty acid; and b, the percentage of the same, theoretically obtainable from the pure salt of the higher fatty acid. Then—

$$Bx = 100P + bx - 100b.$$

For example, suppose a mixed barium salt known or assumed to consist of acetate and valerate to have yielded a precipitate of barium sulphate equivalent 78.45% of the weight taken, when treated with sulphuric acid and ignited. Then, by the above formula,

$$91.37x = 7845 + 68.73x - 6873$$

therefore $22.64x = 972$

and $x = 42.93.$

Hence the mixed barium salt consisted of 42.93 of barium acetate, and 57.07 of barium valerate. From these data and the weight of mixed barium salt found, the actual amounts of acetic and valeric acid may be calculated.

The above method was proposed by Dupré for approximately determining the fusel oil in spirits. In this case the various alcohols are first converted into the corresponding acids by oxidation with chromic-acid mixture.

It has been stated that butyric and valeric acids are extracted from a water solution by shaking with benzene while formic and acetic remain in the water (see *Analyst*, 1908, **33**, 133).

Duclaux (*Ann. Chim. Phys.* [5] 1874, **2**, 289) claimed to have established that each of the lower acids of the formic series has its own rate of distillation, whether alone or mixed with homologues. Several investigators have gone over this method and found it unsatisfactory. H. D. Richmond (*Analyst*, 1895, **20**, 193, 217) examined it very carefully and decided that in the form given by Duclaux it is untrustworthy.

Richmond gives as the result of many experiments the following formula for the distillation of each acid, but it is not established that the formula will apply to any mixture.

$$100 - y = K \frac{(100 - x)^a}{100^{a-1}}$$

In this formula, x, is the percentage of liquid distilled, y the percentage of acid distilled and a and K are factors for each acid ascertained by experiment, as follows (K is practically negligible):

	a	K
Formic (Duclaux)	0.4	1.00079
Acetic (Duclaux)	0.667	1
Propionic (Duclaux)	1.111	1.000723
Butyric (Duclaux)	2	1 (?)
Butyric (Wollny)	2	1
Butyric (Richmond)	2	1
Valeric (Duclaux)	3	1 (?)
Caproic (Duclaux)	4	1.003508
Caprylic (Duclaux)	8 (?)	(?)

For an illustration of the practical application of this method, see a paper by Richmond in *Analyst*, 1908, **33**.

Formic Acid, $HCHO_2$.—Formic acid is contained in the liquid obtained by distilling ants with water. The stings of some insects and plants probably contain it. It is usually prepared by distilling oxalic acid with glycerol. A formate is produced in the decomposition of chloroform or chloral by an alkali, by the reaction of carbon monoxide and alkalies, and of cyanogen gas or cyanides with water.

Formic acid is a colourless volatile liquid, of irritating pungent odour

and very sour. It has a sp. gr. of 1.2211 at 20°, and boils at 100°. It produces intense irritation of the skin.

In general properties it resembles acetic acid, but it is stronger and more readily oxidised.

The *formates* mostly crystallise well and are all soluble in water, Heated with concentrated sulphuric acid they do not blacken, but evolve pure carbon monoxide, as a colourless gas burning with a pale blue flame. A neutral solution of formate gives the following reactions:

Silver nitrate gives, in concentrated solutions, white crystalline silver formate, which darkens on standing, and is reduced to metallic silver when warmed. If the liquid be too dilute to allow of a precipitate being formed, the reduction to metallic silver still occurs on heating, a mirror being frequently formed on the sides of the tube. In presence of ammonium hydroxide the reduction is retarded or prevented.

Mercuric chloride is reduced on heating, with production of white mercurous chloride or grey metallic mercury, according to the proportion of formate present. Acetates do not give this reaction, but acetates and chlorides of alkali metals retard or prevent the reduction. The reduction of mercuric formate on heating may be applied to the estimation of formic acid, and its separation from acetic acid may be approximately effected by boiling the solution of the free acids with yellow mercuric oxide until effervescence ceases. If formic acid only is present, the filtered liquid will be free from mercury. With a mixture of the two acids, the amount of mercury which passes into solution is equivalent to the acetic acid present. If the total acid present originally is determined by standard alkali or other means, the quantity of formic acid may be found, or in presence of other acids forming soluble mercuric salts, the excess of mercuric oxide may be dissolved by dilute hydrochloric acid, and the residual metallic mercury weighed. This weight multiplied by 0.23 will give the weight of formic acid present.

Chlorine, bromine, chromic acid, permanganates and other powerful oxidising agents convert formic acid more or less readily into carbonic acid.

When heated gently with alcohol and sulphuric acid, formates generate ethyl formate, having a fragrant odour of peach-kernels. With ferric chloride, formates react similarly to acetates. At a gentle heat, strong sulphuric acid evolves carbon monoxide from formic acid or a formate. Strong alkalies produce an oxalate.

Lead and magnesium formates are insoluble in alcohol, while the corresponding acetates are soluble. Hence, acetic may be separated from formic acid by saturating the free acids with a slight excess of calcined magnesia or lead carbonate, filtering, evaporating the filtrate to a small bulk, and adding a large proportion of alcohol. Magnesium or lead formate is precipitated, while the corresponding acetate remains in solution. The process may be modified by precipitating the alcoholic solution of the acids with an alcoholic solution of lead acetate, and washing the resultant precipitate with alcohol.

Formic acid may be detected by reduction to formaldehyde. Fenton and Sisson (*Proc. Cambridge Philos. Society*, 1907, **14**, 385) find that this is best accomplished by the action of magnesium in powder or ribbon. A few minutes suffice to produce sufficient formaldehyde for detection by the standard tests. Of course, the absence of formaldehyde must be first established. If it is present, it may be destroyed by pure potassium cyanide as described on page 91. The formic acid can be separated by distilling and the distillate tested.

In addition to the methods already indicated, formic acid may be estimated by titration with standard alkali or by decomposition in a carbonic acid apparatus by sulphuric acid and potassium dichromate, the amount of formic acid present being deduced from the weight of carbon dioxide evolved. 44 parts of carbon dioxide are equivalent to 46 parts of formic acid.

Formic acid and *sodium formate* are used as food preservatives. Woodman and Burrell (*Tech. Quart.* 1908, **21**, 1), have devised the following method for detecting these substances in food.

50 grm. of the sample are mixed with 20 c.c. of 20% phosphoric acid solution, and distilled by means of open steam, the mixture being gently heated to avoid much condensation. A distillate of about 200 c.c. should be collected. Almost all the formic acid is thus obtained. The distillate is mixed with 2 c.c. of 30% acetic acid (free from formic) and 0.2 grm. calcium hydroxide in form of milk of lime. If the distillate is very acid more of the hydroxide may be needed. The solution is evaporated to small bulk over a free flame, and then, on a steam-bath, to dryness. The evaporation should be carried as far as possible over the flame, as the boiling prevents the formation of a crust of calcium carbonate. The dry residue is put into a test-tube provided with a cork and delivery tube. The lower end of the de-

livery tube should dip into about 3 c.c. of water in a tube standing in cold water. The test-tube containing the dry residue is heated gradually to redness, or, at least until vapors are no longer produced. Formic acid or a formate present in the original material will give formaldehyde in the final distillate. W. and B. use the fuchsin test (see p. 257). As a slight reaction is produced by the products of destructive distillation even in absence of formate, they use a color standard prepared by mixing 8 c.c. of copper chloride solution (12 grm. $CuCl_2$, $2H_2O$ in 1000 c.c.) and 12.5 c.c. cobalt chloride solution (24 grm. $CoCl_2$, $6H_2O$ and 100 c.c. strong hydrochloric acid in 1000 c.c.) and diluting this mixture to 100 c.c. Many food products were tested by W. and B., and found not to give a color greater than the standard, while 0.025 grm. of formic acid in 50 grm. of material gave a colour from 4 to 6 times as deep.

Propionic Acid, $HC_3H_5O_2$.—This body, is of little commercial importance, but its detection and separation from its homologues are occasionally necessary.

Propionic acid is contained in crude oil of amber, in sour coconut milk and in certain wines, especially when the fermentation has been pushed too far. It is also produced by the fermentation of glycerol and lactic acid, and by many synthetic methods. It closely resembles acetic acid, but has an odour recalling at once those of acetic and butyric acids. It boils at 140° and has a sp. gr. of 0.996 at 19°.

The *propionates* closely resemble the acetates; they are all soluble in water.

The following method is described by Linnemann for the separation of propionic acid from its lower homologues: The free acids are evaporated to dryness with excess of litharge. The residue is then treated with cold water and the liquid filtered. Basic propionate of lead dissolves, while any acrylate remains insoluble, together with most of the acetate and formate. The solution is boiled and stirred quickly, when the propionate separates suddenly and almost completely as a crystalline precipitate, soluble in cold water, but which may be filtered at a boiling heat from the remaining acetate and formate. The propionic acid of fermentation is said not to exhibit this reaction.

Butyric Acid, $HC_4H_7O_2$.—Two modifications of this acid are known.

Normal butyric acid, $C_3H_7.COOH$, occurs ready-formed in various natural products, and is frequently produced by the decomposition of

animal and vegetable matter. Butyric esters exist in butter and cod-liver oil and it can be produced by a special fermentation of sugar.

Normal butyric acid is a colourless mobile liquid, having a smell at once resembling acetic acid and rancid butter. It is soluble in water, alcohol and ether in all proportions, but is not soluble in concentrated solution of calcium chloride or common salt; hence it may be separated from its aqueous solution by saturating the liquid with calcium chloride and agitating with ether. From the ethereal layer it may be recovered by spontaneous evaporation or, as a salt, by agitation with excess of solution of sodium hydroxide.

For other methods of approximately separating butyric from acetic and valeric acids see page 515.

Isobutyric acid, $CH(CH_3)_2.COOH$, occurs in carob beans and among the acids derived from castor oil. It closely resembles the normal acid in its general properties, but has a lower b. p. and sp. gr. Its smell is less offensive than that of the normal acid obtained by the decomposition of butter or by fermentation of sugar. It requires 3 parts of cold water for solution, and is easily oxidised to acetic acid and carbon dioxide when heated with chromic-acid mixture (page 236).

All *butyrates* are soluble in water. Lead butyrate is a heavy liquid, which solidifies when cooled.

Copper butyrate forms bluish-green monoclinic crystals, which are sparingly soluble in water. The formation of this salt may be employed to distinguish butyric from valeric acid.

The *isobutyrates* closely resemble the butyrates, except those containing calcium and silver. *Normal calcium butyrate* is very soluble in cold water, but separates as a crystalline precipitate on heating the strong solution to 70°. The *isobutyrate* is more soluble in hot water, and separates on cooling as a crystalline magma.

Ethyl butyrate can be formed by heating a butyrate with alcohol and strong sulphuric acid. It has a fragrant odour of pineapple, and boils at 120°.

Ethyl butyrate is produced when butter-fat is saponified by alcoholic solution of a strong alkali. The reaction is easily brought about by adding a small piece of butter (it is not necessary to render out the fat) to some strong solution of sodium hydroxide in alcohol, and heating the mass cautiously until it foams actively. The liquid is then quickly poured into a comparatively large volume of cold water, when the

characteristic odour of the ester is easily noticed. The equation of the reaction is unknown. The test is a convenient one for distinguishing butter from straight butter substitutes, but is, of course, of no value for mixtures containing appreciable amounts of butter-fat.

Valeric Acid; Valerianic Acid; $H_2C_5H_8O_2$.—Four forms of this are possible, derived from the four primary amyl alcohols.

Propyl-acetic Acid; Normal Valeric Acid.—This is obtained by synthetic methods, also from calcium lactate by the action of some fission fungi and by the action of an enzyme contained in the tissues of *Ascarides* on carbohydrates. It has an odour recalling that of butyric acid. It boils at 185° and has a sp. gr. of 0.9415 at 20°.

Methylethyl-acetic Acid.—This can be obtained from the oil of the fruit of the *Angelica archangelica L.*, and probably exists in small amount in valerian root. It is optically active, having the value $[a]_D = 17.85°$. Some synthetic forms are inactive by racemism, but the ordinary form of active amyl aclohol gives the dextrorotatory form of the acid. It boils at about 172°.

Isopropylacetic Acid; Isovaleric.—This is the common form, ordinarily called valerianic acid. It occurs in valerian root. It is optically inactive, but when prepared from valerian root often has slight optical activity, due, it is thought, to a small amount of the active isomer. Esters of this occur in dolphin and porpoise oils, in sweat, and in various other products and secretions of animals. It exists in valerian root and many *Compositæ*. It is a colourless, oily liquid, with an odour resembling old cheese. Its taste is sharp and acid, and it blanches the tongue. It dissolves in about 30 parts of cold water, and is readily soluble in alcohol, ether, chloroform or strong acetic acid. It is almost wholly removed from its aqueous solution by saturating the liquid with common salt or calcium chloride.

This acid has a sp. gr. of 0.937 at 15°, and boils as 175°. It forms a hydrate of the composition $C_5H_{10}O_2,H_2O$, having a density of 0.950 and boiling at 165°, but it is gradually dehydrated by distillation, the weaker acid coming off first. On the other hand, on distilling the dilute aqueous acid, the first portions of the distillate are most strongly acid.

Trimethylacetic acid is solid at ordinary temperatures, melting at 35.4° to a liquid of 0.905 sp. gr. at 50°, and boiling at 163.8°. It is optically inactive.

Reactions of Isovaleric Acid and Isovalerates.—When isovaleric acid or an isovalerate is distilled with sulphuric acid and a little

amylic alcohol, a fragrant ethereal liquid smelling of apples is obtained; this is amyl isovalerate.

Isovalerates are decomposed by acetic acid with formation of isovaleric acid and an acetate; they are also decomposed by tartaric, citric and malic acids.

Isovalerates are mostly soluble in water. Iron and bismuth oxyisovalerates are insoluble. Silver and mercurous isovalerates are but slightly soluble, and aluminum isovalerate is insoluble. Neither this acid nor butyric gives a precipitate with an aqueous solution of zinc acetate. This fact distinguishes them from *caproic acid*, which throws down sparingly soluble zinc caproate as a white crystalline precipitate.

Barium isovalerate crystallises easily in triclinic scales or tables (in distinction from the same compound from active valeric acid), is soluble in 2 parts of cold water and sparingly soluble in alcohol. Barium *caprylate* requires 120 parts of cold water for solution, and is nearly insoluble in alcohol. Barium *caprate* is almost insoluble in water.

When concentrated isovaleric acid is agitated with solution of copper acetate, anhydrous copper isovalerate separates in oily drops which, in from 5 to 20 minutes, crystallise as greenish-blue monoclinic prisms or octohedra of hydrated copper isovalerate, moderately soluble in water and alcohol. The salt is less soluble in hot water than in cold, and hence the saturated solution becomes turbid when heated. This reaction distinguishes the acid from butyric acid, which forms with a moderately strong solution of copper acetate an *immediate* precipitate or turbidity of copper butyrate, of bluish-green colour, and crystallising in small monoclinic prisms. In using this test for assay the acid must first be obtained free by distilling the salt with a moderate excess of sulphuric acid.

Isovaleric acid may be separated from most organic acids by converting it into the soluble lead salt. Acetic acid may be detected by neutrallising any free acid with sodium hydroxide, and precipitating in the cold with excess of ferric chloride. In presence of acetic or formic acid, the filtered liquid will have a red colour. The insolubility of aluminum isovalerate might probably be employed for the separation of the acid from acetic or formic acid.

For other methods of estimating the acid and separating it from its homologues, see page 515.

Commercial Valeric Acid and Valerates.—The presence of *alcohol, acetic acid, butyric acid and valerates*, in commercial valeric acid is indicated by the increased solubility of the sample, which should not be greater than 1 of the hydrated acid in 26 parts by weight of water. If the sample requires more than 30 parts of cold water for solution, the presence of *higher homologues*, or *valeral* (valeraldehyde, $C_5H_{10}O$) is indicated. Acetic acid may be recognised as indicated on page 525. By neutralising the sample with an alkali, any amyl alcohol, valeric aldehyde or neutral ester will be left undissolved, as a turbidity or oily layer, and the amount may be estimated by measurement, or the mixture may be shaken with ether, and the ethereal liquid evaporated spontaneously. The solubility of valeric acid in a mixture of equal volumes of glacial acetic acid and water may be employed to separate it from valeral and esters, but not from amyl alcohol. The presence of butyric acid will be indicated by fractional distillation and by the composition of the salt obtained by saturating the acid with barium carbonate; also by the reaction with copper acetate.

Valeric acid should also be tested for non-volatile impurities, sulphuric acid, and hydrochloric acid.

Valerates have been somewhat extensively used in medicine, especially the sodium, iron, zinc and bismuth salts. They are all more or less liable to adulteration, which in some instances is very gross. Thus, samples of zinc valerate are occasionally composed of the sulphate or acetate, and others have been met with which consisted of zinc butyrate impregnated with oil of valerian. Zinc valerate is also liable to adulteration with tartaric and citric acids, boric acid and other substances. Similarly, iron tartrate or citrate flavoured with valerian has been substituted for the iron valerate, and the quinine sulphate for the valerate. Ammonium valerate has been prepared by saturating calcium chloride with oil of valerian, and many similar frauds have been practised.

Most of the above adulterations may be readily detected. The substitution of zinc butyrate for valerate is best recognised by distilling the salt with sulphuric acid diluted with an equal measure of water, and then applying the copper acetate and other tests to the distillate.

The most satisfactory ready test for valerates is to weigh or measure the layer of free acid which separates on decomposing the solid salt with sulphuric acid diluted with an equal measure of a saturated aqueous solution of zinc sulphate.

Oxalic Acid.

This acid is extensively formed in the physiologic processes of plants and animals. It is usually converted into calcium oxalate, appearing as crystalline deposits (raphides) in cells of plants, but potassium hydrogen oxalate is sometimes found in plant juices. Calcium oxalate is often found in small amount in urine.

Oxalic acid is a product of the action of nitric acid, alkaline potassium permanganate and other oxidising agents on many organic bodies.

On a large scale, the acid is usually made by the action of alkalies starch, sawdust, straw, bran or similar vegetable matter is heated with potassium hydroxide an oxalate is formed. Wheat bran yields 150% of its weight of crystallised oxalic acid. Sodium hydroxide cannot be advantageously substituted, but with a mixture of the alkalies very satisfactory results are obtained. The product of the action is treated with water, and the solution treated with slaked lime. The alkalies are thus recovered. The calcium oxalate is separated and decomposed with sulphuric acid, the resulting acid being separated by evaporation and crystallisation.

Oxalic acid usually occurs crystallised with 2 molecules of water, in monoclinic prisms having a sp. gr. of 1.641 at 4°. Exposed to dry air, or in vacuo over oil of vitriol, the crystals lose water, become opaque, and form a white powder. The acid may be also obtained anhydrous by exposure to a gentle heat (60° to 70°). If at once heated to 100° the crystals melt, and it is then much more difficult to drive off the water. By dissolving ordinary oxalic acid in 12 parts of warm concentrated sulphuric acid, and allowing the solution to stand for several days, the anhydrous acid, is deposited in transparent crystals, which on exposure to air absorb water and fall to powder.

Saturated solutions of oxalic acid lose acid at 100°, and the anhydrous acid may be readily sublimed. This furnishes a convenient mode of obtaining the pure acid for analytic purposes. The acid should previously be rendered anhydrous by heating to 60° or 70°, and the temperature of the retort must be kept as constantly as possible at 157°. If allowed to rise to 160°, much loss of acid occurs, and an inferior product is obtained containing water and formic acid. The passage of a current of dry air greatly facilitates the sublimation.

Oxalic acid is colourless and odourless, and completely volatile by heat without charring.

100 parts of water dissolve 8 parts of crystallised oxalic acid at 10° and 345 parts at 90°. The solution is intensely sour, reddens litmus strongly, and is very poisonous. It decomposes carbonates, phosphates, chromates and many other salts, including fluorspar. Powdered oxalic acid completely decomposes sodium or calcium chloride when the mixture is heated. Prussian blue dissolves in oxalic acid to a clear blue liquid, sometimes employed as a blue ink. Solutions of oxalic acid are permanent in the dark, but when exposed to light the acid is rapidly decomposed.

Crystallised oxalic acid dissolves readily in cold and still more readily in boiling alcohol. It is but slightly soluble in ether, and is insoluble in chloroform, benzene or petroleum spirit.

Oxalic acid is not affected by boiling with moderately strong nitric or hydrochloric acid. Cold sulphuric acid has no action on it; but when heated with concentrated sulphuric acid, it decomposes into carbon monoxide, carbon dioxide and water.

When heated with glycerol, oxalic acid yields carbon dioxide and water at a moderate heat and formic acid at a higher temperature. This is the method commonly employed for producing formic acid.

Manganese and lead dioxides convert oxalic acid in carbon dioxide and water. Auric chloride and acid solutions of permanganates react similarly.

Reactions of Oxalic Acid and Oxalates.—An aqueous solution of oxalic acid presents the following analytical characters:

On addition of lime-water or solution of calcium acetate, a white precipitate of calcium oxalate is formed. The precipitate is insoluble in water, and not sensibly soluble in acetic or other organic acids. It is readily soluble in dilute mineral acids. It is decomposed by boiling with excess of sodium carbonate solution, with formation of insoluble calcium carbonate and soluble sodium oxalate. On gentle ignition, calcium oxalate evolves carbon monoxide and leaves calcium carbonate. No blackening occurs. Solutions of soluble oxalates give the same reaction as oxalic acid with lime-water or calcium acetate, and react with calcium sulphate or chloride in addition. If previously neutralised by ammonium hydroxide, oxalic acid solutions are precipitated by the two latter reagents.

With solutions of barium, oxalic acid and oxalates react in a similar manner as with solutions of calcium, but the resultant barium oxalate is not so insoluble in water or acetic acid as the calcium salt.

On addition of dilute sulphuric acid and manganese dioxide, warm solutions of oxalic acid and oxalates produce effervescence, owing to the formation of carbon dioxide. The gas may be proved to be carbon dioxide by its reaction with lime-water.

In presence of dilute sulphuric acid, a warm solution of oxalic acid rapidly decolourises potassium permanganate. From strong solutions, the resultant carbon dioxide escapes with effervescence.

Estimation of Oxalic Acid.—Oxalic acid may be estimated with considerable accuracy by either of the following methods, the details of which may be found in most works on quantitative analysis:

a. By precipitation as calcium oxalate. The solution should be hot and dilute, and mineral acids must be absent or previously neutralised by ammonium hydroxide. In the absence of other acids forming insoluble or nearly insoluble calcium salts (*e. g.*, sulphates, tartrates, citrates, phosphates), the solution may be exactly neutralised by ammonium hydroxide, and calcium chloride added. Any phosphate may be separated by digesting the precipitate with cold dilute acetic acid. In presence of sulphates, calcium sulphate should be employed as a precipitant. It is frequently preferable to have the solution acid with acetic acid or to precipitate the acid solution with calcium acetate, so as to avoid the precipitation of other calcium salts. Almost all calcium salts are soluble in acetic acid, except the oxalate, racemate, and fluoride. Racemates may be previously removed by precipitation with potassium acetate in presence of alcohol. The separation of oxalates and fluorides is rarely required in practice, but, if required the oxalate can be determined by titrating the precipitate with standard potassium permanganate. The precipitate of calcium oxalate, however produced, is to be well washed and then treated in one of the following ways:

1. It is dried at 100°, and weighed as calcium oxalate.

2. It is ignited, moistened with ammonium carbonate, again gently ignited, and weighed as calcium carbonate.

3. It is moistened on the filter with strong sulphuric acid, and the whole ignited again, moistened with sulphuric acid, reignited, and finally weighed as calcium sulphate.

4. It is ignited thoroughly, and the resultant calcium oxide and carbonate titrated with standard acid.

5. The filter is placed in a beaker together with water and dilute

sulphuric acid, and the liquid is titrated with standard potassium permanganate.

Of these methods, the last two are perhaps the best, because they are the least affected by impurity in the precipitate. Process 5 aims at the direct estimation of the oxalate, and may be applied to a precipitate containing phosphate, carbonate, or sulphate; but tartrate, racemate, and most organic salts must be absent from the precipitate.

b. By treatment with dilute sulphuric acid and manganese dioxide in a carbon dioxide apparatus. This process is conducted precisely as in the valuation of a manganese ore, except that excess of manganese dioxide is used instead of excess of the oxalate. 44 parts by weight of carbon dioxide lost by the apparatus represent 63 of crystallised, or 45 of anhydrous oxalic acid.

c. By titration with standard permanganate. The solution of the oxalate must be free from other readily oxidisable bodies, and should be warm, dilute, and pretty strongly acidulated with sulphuric acid. The permanganate is added gradually, with constant stirring, until the liquid acquires a permanent pink tint. The permanganate is preferably standardised with pure oxalic acid. N/10 potassium permanganate, is a suitable strength. Each c.c. of this solution will oxidise 0.0063 grm. of crystallised or 0.0045 grm. of anhydrous oxalic acid. The process can be employed for titrating a precipitate of calcium oxalate.

In cases of poisoning by free oxalic acid, the acid extracted from the stomach and intestines is chiefly uncombined, but that obtained from the liver, kidneys, heart and urine is in combination.

Commercial oxalic acid is not much liable to intentional adulteration.

Organic matters other than oxalic acid are recognised by the charring or darkening of the sample when heated, or on warming with concentrated sulphuric acid.

Fixed mineral impurities are left as a residue on igniting the sample in the air. If the ignited residue effervesces on addition of dilute acid, an *acid oxalate* is probably present in the sample. Sensible quantities of *lead* and other heavy metals are sometimes met with. *Sulphuric acid and acid sulphates* are sometimes present in considerable amount. The solution of such samples gives a white precipitate with barium chloride. The same impurities occur in commercial ammonium oxalate.

Oxalates.—These salts require but little special description. The metals of the potassium group form 3 classes of oxalates, the potassium salts having the formulae $K_2C_2O_4,H_2O$;KHC_2O_4,H_2O; and $KH_3(C_2O_4)_2,2H_2O$. The acid salts are the least soluble. The oxalates from most other metals are insoluble, or nearly insoluble, in water. This is true of the oxalates from barium, strontium, calcium, copper, magnesium, manganese, cobalt, nickel, zinc, lead and silver. The first 4 of these retain 1 molecule of water on drying at 100°. The remainder retain 2 molecules, with the exception of the lead and silver salts, which are anhydrous. Ferrous oxalate is but sparingly soluble, but ferric oxalate is readily so, at least in presence of free oxalic acid; hence the use of oxalic acid for removing ink-stains and dissolving Prussian blue. All the insoluble oxalates are soluble in dilute nitric acid, but they are generally insoluble in acetic acid. The estimation of the oxalic acid may be readily effected by the methods described on page 529.

On ignition, oxalates containing metals not easily reducible evolve carbon monoxide, and leave carbonates. These may sometimes be further decomposed if the temperature be excessive. Oxalates containing more easily reducible metals, when heated to redness in a close vessel, usually leave the metal and evolve carbon dioxide. This reaction occurs even at 100°, in the case of gold; hence gold is reduced from its solutions by boiling with an oxalate.

Pure oxalates do not char on ignition.

Succinic Acid.—Succinic acid occurs naturally in amber and in certain lignites; is produced during the alcoholic fermentation of sugar; and by the fermentation of malic acid and many other substances, especially under the influence of putrefying casein; also by the action of nitric acid on the fatty acids and fats, and it exists ready formed in several plants.

It may be obtained by the dry distillation of amber, the watery distillate being filtered while hot to separate oil, when crystals of the acid are deposited on cooling, and may be purified by boiling with nitric acid, followed by recrystallisation from water.

Succinic acid bears the same relation to butylene (tetrene) alcohol that oxalic acid does to ethylene glycol, and may be produced from butylene alcohol by oxidation. It may also be obtained by the deoxidation of tartaric or malic acid, which contain, respectively, 2 and 1 atom more of oxygen than does succinic acid.

Succinic acid crystallises in colourless, oblique rhombic prisms or plates. When heated to 130°, it emits suffocating fumes, and at 180° melts. When the heat is increased to 235° the acid boils and sublimes as succinic anhydride, which melts at 120°. When heated strongly in the air, succinic acid burns with a blue smokeless flame.

Succinic acid is soluble in about 18 parts of cold and 0.8 boiling water. It dissolves readily in alcohol and sparingly in ether, but is insoluble in chloroform, benzene, petroleum spirit, turpentine or carbon disulphide. Nitric acid, chlorine and chromic acid have no action on succinic acid, and it is soluble without change in strong sulphuric acid. Permanganates have no action on a cold acid solution, but when heated in presence of free alkali produce oxalic acid.

Reactions of Succinic Acid.—In its analytical characters succinic acid somewhat resembles benzoic acid, but differs from it in not being precipitated from a strong solution of its salts by hydrochloric acid; in being precipitated by ammoniacal solution of barium chloride even from a dilute solution; and by being insoluble in chloroform, and therefore not removable from an acid solution by agitation with that liquid. Magnesium benzoate is soluble in alcohol, but the succinate is insoluble.

Ferric chloride, if first treated with as much ammonium hydroxide as it will bear without precipitation, will throw down from neutral solutions of soluble succinates a bulky cinnamon-brown basic ferric succinate, some free succinic acid being simultaneously produced, and the solution acquiring an acid reaction. Benzoates, under similar circumstances, give a flesh-coloured precipitate, and cinnamates a yellow. The precipitate may be filtered off, washed and decomposed by boiling with excess of dilute ammonium hydroxide. The filtered liquid, if mixed with barium chloride and an equal bulk of alcohol, gives a white precipitate of barium succinate. By the above combination of reactions, succinic acid may be readily identified and separated from other organic acids. The process might possibly be made quantitative. For such a purpose, sodium acetate should be added to the liquid containing the iron precipitate, and the whole boiled, the precipitate produced being first boiled and then washed with dilute ammonium hydroxide, the liquid being then concentrated and precipitated by alcohol and barium chloride. Neutral succinates containing alkali metals may also be precipitated pretty completely by adding barium chloride to the boiling solution.

Commercial succinic acid has usually more or less of a brown colour, and somewhat of the odour of empyreumatic oil of amber, which impurity may be removed by agitation with petroleum ether. A *factitious succinic acid* has been prepared by adding a little oil of amber to tartaric acid, ammonium chloride or potassium hydrogen sulphate.

Inorganic impurities and adulterants will be left on igniting the substance. *Cream of tartar* leaves potassium carbonate on ignition; it has been found in succinic acid to the extent of 50%. Barium sulphate may be recognised by its insolubility and other characters; and boric acid by the reddish-brown colour imparted to turmeric paper, when the ash is acidulated with hydrochloric acid and the solution evaporated in contact with it. Heavy metals may be recognised by the usual tests.

Foreign organic acids may be detected by their special reactions. Thus *oxalic acid* will be precipitated on adding calcium acetate (or a mixture of calcium chloride and ammonium acetate) to the aqueous solution of the sample; *tartaric acid* by potassium acetate and alcohol; *citric acid* by the precipitate formed on adding excess of lime-water and boiling. *Benzoic acid* may be detected by its solubility in carbon disulphide or warm petroleum spirit, and by its separation on treating the precipitate produced in the neutralised liquid by ferric chloride with hydrochloric acid.

Ammonium chloride may be recognised by the tests for ammonium salts and chlorides.

Sugar and various other impurities cause charring on warming the substance with sulphuric acid.

A useful method of examining succinic acid is to dissolve 1 grm. of the sample in 15 c.c. of hot alcohol in which it should be completely soluble. When cold, one-half the solution is mixed with an equal volume of chloroform, and the other with an equal measure of ammonia. Complete admixture should occur in both cases. If the result of the test is satisfactory, and the sample leaves no sensible quantity of ash, and does not notably darken with strong sulphuric acid, the substance may be considered pure.

Malic Acid.

Malic acid is contained in apples, pears and many fruits used for domestic purposes. It is usually prepared from rhubarb stalks or mountain-ash berries.

Malic acid crystallises in groups of 4- or 6-sided prisms, which are colourless and odourless, and readily fusible. Malic acid is deliquescent and readily soluble in water, alcohol and ether. The aqueous solution has an agreeable acid taste, and becomes mouldy on keeping. In contact with ferments, especially putrid cheese, the solution of malic acid yields succinic and acetic acids and sometimes butyric acid.

When heated in a small retort to about 180°, free malic acid melts and evolves vapours of maleic and fumaric acids, which crystallise on the cooler parts of the retort and receiver. Fumaric acid, forms slowly at 150°, and mostly crystallises in the retort, in broad, colourless, rhombic or hexagonal, prisms, which vapourise without melting at about 200°, and are soluble in 250 parts of cold water, and easily in alcohol and ether. Maleic acid is the chief product if the temperature be suddenly raised to 200°. This body crystallises in oblique rhomboidal prisms, which melt at 130°, vapourise at about 160°, and are readily soluble in water and alcohol. The behaviour of malic acid on heating is of value owing to the few characteristic tests for this acid. Maleic and fumaric acids are stereo-isomers.

Malic acid, exhibits optical activity. It exists in two forms: dextrorotatory and lævorotatory.

By the action of hydriodic acid, under pressure, malic acid is converted into succinic acid. Nitric acid and alkaline solutions of permanganate oxidise malic acid. Concentrated sulphuric acid darkens malic acid and malates very slowly on warming. When boiled with dilute sulphuric acid and potassium dichromate, malic acid evolves an odour of ripe fruit.

No malate is quite insoluble in water; only a few are soluble in alcohol. Solution of calcium chloride does not precipitate malic acid or malates in the cold (distinction from oxalic and tartaric acids); only in neutral and very concentrated solutions is a precipitate formed on boiling. (Citrates are precipitated from neutral boiling solutions by calcium chloride, unless the liquid is very dilute.) The addition of alcohol after calcium chloride produces a bulky, white precipitate of calcium malate, even in dilute neutral solutions. Thus, if the liquid be filtered first cold (to remove oxalic and tartaric acids), and then boiling hot (to remove citric acid), the malic acid can be precipitated on addition of 2 volumes of alcohol. This precipitate may contain calcium sulphate or succinate, but will be free from formate, acetate, benzoate except that if more than 2 volumes of alcohol are added, cal-

cium formate precipitate. On boiling the precipitate with a moderate quantity of water, the malate will be dissolved, and tannate and sulphate left almost wholly behind. The precipitate produced by calcium chloride and alcohol may also be tested for malic acid (after drying it to get rid of all trace of alcohol) by decomposing it with dilute sulphuric acid, and boiling the filtered liquid with a *small* quantity of potassium dichromate. If the liquid remains yellow, succinic acid alone is likely to be present; but if green and without odour, citric acid is probably present either with or without succinic acid. If the liquid becomes green and evolves an odour of ripe fruit, malic acid is present, and possibly either or both succinic and citric acid in addition.

Solution of lead acetate precipitates malates, more perfectly after neutralisation with ammonia, as a white (and frequently crystalline) precipitate of lead malate, which, on boiling for a few minutes, sets under the liquid to a transparent, waxy, semi-solid. This characteristic reaction is obscured by the presence of other organic acids. The precipitate is very sparingly soluble in cold water, somewhat soluble in hot water. Lead malate is soluble in strong ammonia, but is not readily dissolved by a slight excess. (Distinction from tartrate and citrate.) It dissolves in ammonium acetate, and on mixing the liquid with 2 volumes of alcohol is reprecipitated. (Lead succinate remains in solution.)

The precipitate of lead malate may be washed with a mixture of 2 volumes of alcohol and 1 of water.

If the precipitate of lead malate is treated with excess of ammonium hydroxide, dried on the water-bath, moistened and triturated with alcoholic ammonia, and then treated with absolute alcohol, only ammonium malate dissolves; ammonium citrate, tartrate, and oxalate, being insoluble in absolute alcohol. Malic acid may be separated from other organic acids in solution by adding ammonium hydroxide in slight excess, and then 8 or 9 volumes of strong alcohol, which precipitates all but the ammonium malate. The method may be conveniently applied to the solution of the acids obtained by suspending the lead salts in water and passing hydrogen sulphide through the liquid.

If the alcoholic solution of ammonium malate is precipitated by lead acetate, and the lead malate obtained filtered off, washed with alcohol, dried at 100° and weighed, the weight obtained, multiplied by 0.3953, gives the quantity of malic acid present.

For the estimation of malic acid in wine, see page 187; for estimation. in vinegar see page 505.

Tartaric Acid,

Tartaric acid occurs in some plant juices. Grape juice is the only important source. The deposit formed on the sides and bottom of the vessels in which wine is manufactured consists largely of calcium and potassium tartrates. After purification, it is treated with calcium carbonate and calcium sulphate, by which a nearly insoluble calcium tartrate is produced, and this, when decomposed with sulphuric acid, yields free tartaric acid, which is obtained in crystals by cooling the concentrated liquid.

Three distinct forms of tartaric acid exist. Their chief physical and chemical differences are as follows:

Dextrotartaric, ordinary tartaric acid, forms anhydrous, hemihedral, monoclinic crystals, the aqueous solution of which turns the plane of polarisation to the right, the value for $[a]_D$ at 16° being 13.1° for a 15%, and 14.7° for a 2% solution. The crystals fuse at 135°, have a sp. gr. of 1.74 to 1.76, and are readily soluble in absolute and dilute alcohol.

In the following article this acid and its salts are always referred to unless otherwise stated.

Lævotartaric acid forms anhydrous crystals, the aqueous solution of which turns the plane of polarisation of a luminous ray to the left, the rotation being equal and opposite to that produced by dextro-tartaric acid.

Inactive, or mesotartaric acid, is produced by prolonged heating of dextrotartaric acid to 165° with a small proportion of water. It is optically inactive, but unlike racemic acid is not resolvable into 2 acids. Mesotartaric acid is very soluble in water, forms crystals containing 1 molecule of water, and yields calcium and potassium hydrogen salts more soluble than the corresponding salts of ordinary tartaric acid.

Racemic acid, often described as a fourth form of tartaric acid, is really an association of equal quantities of the active forms and is optically inactive. It can be separated into the two forms and, can also be obtained by mixing equal amounts of them. It occurs with ordinary tartaric acid in crude tartars. It forms crystals containing 1 mol. of water, which effloresce in the air, and become completely anhydrous at 100°; the resultant anhydrous acid melts at about 200°. Racemic acid is soluble in 5 parts of cold water, and with difficulty

in cold alcohol. The calcium racemate is less soluble in water than calcium dextrotartrate, and is also distinguished by its insolubility in acetic acid and in ammonium chloride solution.

The slighter solublitiy of calcium racemate as compared with calcium dextrotartrate has led to the suggestion of a method for detecting the latter by adding a solution of lævotartaric acid to the liquid to be tested, then calcium chloride and neutralizing the solution. The lævotartaric acid will associate with an equal portion of dextrotartaric acid, if any is present, and the highly insoluble calcium salt will precipitate.

Ordinary tartaric acid is soluble in 0.7 part of cold and 0.5 part of boiling water; in 1.6 parts of cold alcohol (95%) and in about 0.2 part of boiling alcohol; in 250 parts of ether, and is nearly insoluble in chloroform, benzene and petroleum spirit.

The following table by H. Schiff shows the sp. gr. of aqueous solutions of tartaric acid:

Percentage by weight of tartaric acid.	sp. gr. at 15°
33	1.1654
22	1.1062
14.67	1.0690
11	1.0511
7.33	1.0337
3.67	1.0167

Unsterilized aqueous solutions of tartaric acid (especially when dilute) gradually decompose on account of the growth of mold. The change may be prevented by the addition of a little phenol. Many tartrates decompose when kept in a moist state.

Most oxidising agents convert tartaric into formic acid. Ammonio-silver nitrate is reduced with formation of carbonic and oxalic acids. In dilute solution, tartaric acid reduces gold and platinum chlorides, and converts mercuric chloride into calomel.

Detection and Estimation of Tartaric Acid and Tartrates.— Tartaric acid and tartrates are charred when heated with concentrated sulphuric acid of 1.845 sp. gr. The reaction may be used to distinguish a tartrate from a citrate or to detect tartaric acid in presence of citric acid. For this purpose, 1 grm. of the sample should be treated with 10 c.c. of pure concentrated sulphuric acid (free from nitrous com-

pounds), and the mixture heated to 100° for 40 minutes. Citric acid gives only a yellow colour when thus treated, but if 1% of tartaric acid be present the liquid has a distinct brown shade, and this becomes still more marked with larger proportions.

If a drop of ferrous sulphate solution is added to a solution of tartaric acid or soluble tartrate, then a few drops of hydrogen peroxide, and the mixture finally treated with excess of sodium hydroxide, a fine violet is produced, which in strong solutions is so deep as to appear almost black. The colour is discharged by sulphurous acid. If potassium ferrocyanide is added to the violet liquid, and then sufficient dilute sulphuric acid to acidify the solution, the iron may be filtered off and a colourless filtrate obtained which again gives the violet colour on addition of a ferrous salt. The colourless filtrate reduces silver and mercury compound, potassium dichromate and permanganates. After adding excess of alkali it precipitates cuprous oxide from Fehling's solution in the cold; on heating, metallic copper is separated.

Acid solution of a permanganate or sodium hypochlorite may be substituted for the hydrogen peroxide in the foregoing test, if care be taken to avoid excess, but the result is not so satisfactory. Heavy metals and oxidising agents must be absent. Citric, malic, succinic, oxalic and acetic acids and sugar were found by H. J. H. Fenton, the observer of the reaction, to give no similar colouration (*Chem. News*, 1876, 33, 190; 1881, 43, 110).

Soluble tartrates in neutral solution give white calcium tartrate on addition of calcium chloride. The precipitate is nearly insoluble in cold water; soluble in many ammonium salts; soluble (after washing) in a cold solution of sodium hydroxide, but reprecipitated on boiling; soluble in acids (including acetic); and converted by heating with a neutral solution of copper chloride into insoluble copper tartrate. Calcium citrate yields soluble copper citrate. Calcium tartrate may also be conveniently examined by dissolving it in the smallest possible quantity of acetic acid, adding excess of potassium-chloride solution and stirring vigorously, when the potassium hydrogen tartrate will be thrown down.

The reducing action of tartaric acid on silver compounds is a delicate test, but is liable to failure if certain conditions are not observed. The solution of tartaric acid, or alkali-metal tartrate (all other metals being first removed), is rendered acid with nitric acid, *excess* of silver nitrate added, and any precipitate filtered off. To the solution, *very*

dilute ammonium hydroxide is added until the precipitate at first formed is nearly redissolved. The solution is again filtered, and the filtrate heated nearly to boiling for a few minutes, when a brilliant mirror will be formed on the sides of the tube. Citric acid does not reduce silver under similar circumstances, but gives a precipitate on continued boiling.

Tartaric acid prevents the precipitation of many metallic solutions by alkalies, stable double tartrates being formed. For the separation of heavy metals from tartrates, hydrogen sulphide or sodium sulphide must be employed, according to the metals present. The filtrate may be concentrated, and any barium, strontium, calcium or magnesium present thrown down by boiling with sodium carbonate. Aluminum is not separated by either of the above precipitants, but the tartaric acid can be detected and estimated in the solution without removing it.

The best method of direct estimation of tartaric acid is to precipitate it in the form of potassium hydrogen tartrate. When the free acid is to be estimated, either alone or mixed only with citric acid, the method described under Citric Acid should be employed. For the estimation of tartaric acid in tartrates and in the various natural and artificial products of tartaric acid manufactories, processes are given below.

Tartaric acid in wine may exist in the free state, and as calcium and potassium hydrogen tartrates, and ethyl tartrate is probably often present. (See page 177.)

Like the corresponding salts of other organic acids, tartrates containing metals not easily reducible, leave on gentle ignition a residue of carbonate or oxide and by dissolving this residue in standard acid and ascertaining the amount of acid neutralized by titrating the excess with standard alkali, an accurate estimation can be effected, and, if it is known whether the tartrate was originally acid or neutral an estimation of the acid itself is obtained.

Tartaric acid and hydrogen tartrates neutralise alkalies completely.

The tartaric acid in *tartrates containing organic bases* may generally be ascertained by precipitation as potassium hydrogen tartrate.

The alkyl tartrates are unimportant. Ethyl tartrate may be decomposed by heating with alcoholic sodium hydroxide and potassium hydrogen tartrate precipitated by adding excess of acetic acid.

Commercial tartaric acid is liable to contain the same impurities

as citric acid, and is examined, in a similar manner. It may be adulterated with alum and potassium hydrogen sulphate, the presence of either of which would be indicated by the ash left on ignition and the formation of a precipitate on addition of barium chloride to the aqueous solution.

Tartaric acid liquors are the liquids resulting from the decomposition of calcium tartrate by sulphuric acid. They are of a very complex character, containing: free tartaric acid; foreign organic acids; sulphuric acid, and calcium, potassium, iron and aluminum sulphates; phosphates; and bodies of an indefinite nature. The analytic examination usually includes estimation of the tartaric and free sulphuric acid, with the additional estimation, in some cases, of the total organic acids.

The estimation of the *tartaric acid* is best effected by precipitation as potassium hydrogen tartrate. Potassium acetate is the best reagent for pure liquors, but it is inapplicable in presence of iron or aluminum. Potassium citrate is free from this objection. It is obtained by neutralising citric acid by pure potassium carbonate or hydroxide and is best employed in the following manner:

A quantity of liquor, of 30 to 40 c.c. in volume, as cold as possible, and containing from 2 to 4 grm. of tartaric acid, is treated with a saturated aqueous solution of the citrate, added drop by drop with constant stirring. As soon as the free sulphuric acid is neutralised the precipitate begins to appear in streaks on the sides of the glass. In presence of much sulphuric acid, a fine precipitate of potassium sulphate will precede the formation of the tartrate, but is readily distinguished therefrom. When the streaks begin to appear, 1 c.c. of citrate solution is added for every grm. of tartaric acid supposed to be present. A great excess should be avoided. Should a gelatinous precipitate be formed, the experiment is repeated with a previous addition of some citric acid. After stirring continuously for 10 minutes, the precipitate is washed 2 or 3 times with 25 c.c. of a 5% solution of potassium chloride, saturated with potassium hydrogen tartrate. The precipitate is then collected on a small filter and washed with the same solution, until the acidity of the filtrate is only slightly in excess of that of the solution used for washing the precipitate. The filter and precipitate are finally transferred to a beaker, and the amount of tartaric acid present is determined by titration with standard alkali set against potassium hydrogen tartrate; litmus or phenolphthalein being used as

the indicator. The presence of potassium sulphate in the precipitate is of no consequence, as it has no neutralising power.

. Sometimes, however, a potassium hydrogen citrate is carried down by the tartrate and obstinately retained. It is best got rid of by dissolving the precipitate in 50 c.c. of hot water, adding 5 grm. of potassium chloride, and cooling the liquid quickly to 15°, stirring continually, and continuing the agitation for 10 minutes. This purified precipitate may be washed with the ordinary washing fluid with great ease, but a correction of 1/2 % on the tartaric acid found must be made for unavoidable loss in the process of purification. The filtrate may be tested for citric acid by neutralising it with sodium hydroxide and adding calcium chloride. After prolonged standing in the cold and filtration from a little calcium tartrate, the solution is boiled, when any precipitate will consist of calcium citrate.

Under favourable circumstances, assays by the above method show from 99 to 100% of the tartaric acid present, but greater differences occur if the proper proportion of citrate is not used. Grosjean concluded that, when an accurate assay of factory tartaric acid liquors is required, a preliminary series of experiments was necessary to ascertain what volume of citrate solution gave a precipitate of maximum acidity. This having been ascertained, a final experiment should be made, using the proper quantity of citrate solution, and washing the precipitate very thoroughly. In presence of much sulphuric acid, the results have a tendency to be in excess of the truth. From very old bad liquors, potassium alum may be precipitated on adding the citrate solution, owing to the formation of potassium sulphate and the sparing solubility of alum in solutions of that salt. When alum has been precipitated the results will be below the truth, as on washing with the potassium chloride solution a fluid is formed in which potassium hydrogen tartrate is readily soluble. If, on the other hand, an alcoholic washing liquid be substituted, the alum is retained in the precipitate, and increases the final acidity. The difficulty may be avoided by adding phosphoric acid before the citrate solution, but the filtration must be effected immediately after the stirring, or a gelatinous precipitate of aluminum phosphate may be thrown down.

Racemic acid, if present, will be estimated as tartaric acid by the above method. Inactive tartaric acid is only imperfectly precipitated, owing to the greater solubility of potassium salt. Oxalic acid has been detected in old liquors, but does not interfere with the results.

The estimation of the *free sulphuric acid* in tartaric acid liquors is troublesome, owing to the insolubility of potassium and calcium tartrates in alcohol and the occasional presence of alum. Thus, it mixed solutions of potassium alum and tartaric acid are treated with alcohol, potassium hydrogen tartrate and alum are precipitated, and the liquid contains sulphuric acid, which was not present originally. A similar reaction occurs if calcium sulphate is substituted for the alum. These errors are removed when the quantity of sulphuric acid in the liquor is sufficiently great, and will occur in practice merely in the case of new liquors of bad quality. (For analytic process see p. 549.)

A useful indication of the presence of sulphuric acid in tartaric acid liquors is obtained by treating the liquid with half its measure of a saturated aqueous solution of calcium chloride. A turbidity due to calcium sulphate occurs immediately in a liquor containing sulphuric acid equivalent to 0.8% of brown oil of vitriol, and in 5 minutes when only 0.1% of oil of vitriol is present.

For the estimation of the *total organic acids* in tartaric acid liquors, R Warington recommends the following method (*Jour. Chem. Soc.*, 1876, 28, 982: Exactly neutralise a known measure of the liquor with standard caustic alkali, evaporate to dryness, and ignite the residue at a very low temperature till the carbon is nearly consumed. Treat the ash with a known quantity of standard sulphuric acid, heat and decant, and treat the insoluble residue with more standard acid, concentrating, if necessary, to effect solution of the phosphates. Treat the mixed cold concentrated solutions with sufficient potassium sodium tartrate to keep any aluminum in permanent solution, and then titrate the solution with standard alkali and litmus. The amount of standard sulphuric acid neutralised *by the ash* is the exact equivalent of the total organic acid in the liquor taken, and each c.c. of normal acid neutralised represents 0.075 grm. of organic acid, expressed in terms of tartaric acid.

Lees; Argol; Tartar.—These are products of the fermentation of grape-juice; they consist largely of potassium hydrogen tartrate and are the materials from which tartaric acid and tartrates are obtained. Their separation is due to the diminished solubility of the tartrates in the alcoholic liquid produced by the fermentation.

Lees is the solid matter collected from the bottom of the vessels in which the grape-juice is fermented.

Its composition is greatly altered by "plastering" the wine. This process consists in adding to the wine an impure calcium sulphate containing some carbonate. "Spanish earth," a kind of readily decomposed clay, is sometimes employed. The result is, that in plastered lees the tartrate exists chiefly as the calcium tartrate instead of the acid potassium salt. The total tartaric acid in lees is usually from 24 to 32%. Lees contain from 30 to 40% of indefinite vegetable matter, the remainder being tartrates, sulphates (in plastered lees), ferric oxide alumina, phosphates and sometimes lumps of plaster.

Argol, or crude tartar, is the crystalline crust deposited on the sides of the vessels used for the fermentation. It exhibits some irregularity of composition, the tartaric acid ranging from 40 to 70%, most of it as potassium hydrogen tartrate. Very low argols resemble superior lees, while first-class argols are equal to ordinary refined tartar. The term "argol" is also applied loosely to both tartar and lees. In argol, globules of sulphur are sometimes found; they are due to the sulphur burnt in the casks before introducing the wine.

Cream of tartar, or refined tartar, is prepared by boiling crude tartar (argol) with water, filtering and crystallising the salt from the clear liquid. The term cream of tartar is derived from the fact that during the evaporation of the liquid the salt collects in white crystalline crusts on the surface of the solution. Cream of tartar consists chiefly of potassium hydrogen tartrate, but contains more or less calcium tartrate, which, though nearly insoluble in pure water, dissolves with moderate facility in a hot solution of potassium hydrogen tartrate. The proportion of calcium tartrate usually present in commercial cream of tartar ranges from 2 to 9%; proportion in excess of 10% may be considered as an adulterant (see a paper by Allen, *Analyst*, 1880, 5, 114). Commercial cream of tartar is adulterated to a considerable extent, the potassium and calcium sulphates, marble, alum and barium sulphate, starch and calcium phosphate being among the substances used, and potassium hydrogen sulphate is sold under the name of "tartalie," and employed as a substitute for cream of tartar. It has a higher neutralising power than cream of tartar, and hence is sometimes diluted with potato starch, the mixture being sold under misleading names. Powdered alum is often sold under the term C. T. S. (cream of tartar substitute).

Assay of Tartar and Argol.—For the *detection of adulterants* in cream of tartar, the following tests may be applied:

The sample should be ignited, the residue boiled with water, filtered off, washed, ignited, moistened with ammonium carbonate, gently re-ignited and weighed. The "insoluble ash" thus obtained from genuine cream of tartar consists of the calcium carbonate corresponding to the *calcium tartrate* originally present, and its weight may be calculated to its equivalent of the latter by multiplying it by the factor 1.88. The calcium tartrate thus found should not exceed 10%, or 12% at the outside. Any higher proportion is usually due to adulteration with calcium compounds. Addition of *calcium chloride* is said to have occurred, though improbable, but there are authentic cases of adulteration by *chalk* and *marble*. Allen found 20% of *calcium sulphate* probably added as *plaster of Paris*. In the case of adulterated samples, the proportion of calcium tartrate cannot be deduced with accuracy from the percentage of "insoluble ash."

The sample is boiled with a moderate excess of pure sodium carbonate and the liquid filtered. A portion of the filtrate is tested for *sulphates* (*e. g.*, calcium sulphate, potassium sulphate and alum) by acidulating slightly with hydrochloric acid and adding barium chloride, and another for *chlorides* by rendering it acid with nitric acid, and adding silver nitrate; traces of sulphates and chlorides may be neglected. The precipitate produced by sodium carbonate should be rinsed off the filter and treated with dilute hydrochloric acid. Any insoluble residue may consist of *sand* or *barium sulphate*. Both the chemical and microscopical characters may be employed to distinguish these, and to determine whether the latter adulterant is crystalline or amorphous.

The presence of *alum* is indicated by the detection of a notable quantity of sulphates, and the presence of aluminum oxide in the insoluble ash. Aluminum hydroxide cannot be precipitated by adding ammonium hydroxide to the original solution of the substance, owing to the presence of tartrate; but it may be detected by neutralising the hot solution of the sample with sodium hydroxide, and boiling the liquid with a little acetic acid and excess of sodium phosphate. Any aluminum present will be thrown down as phosphate, tartrates having scarcely any solvent action on the precipitate at the temperature of ebullition, and in presence of excess of phosphoric acid. Alum may be dissolved out of cream of tartar by treating the finely powdered sample with a cold, saturated, aqueous solution of potassium hydrogen tartrate, containing 5% of potassium chloride.

Starch is easily detected by microscopic examination and the iodine test. For estimation see under "Starch."

Calcium phosphates are detected and determined by treating 0.5 grm. with excess of moderately strong nitric acid, and precipitating with ammonium molybdate in the usual way.

Assay of Crude Tartars.—The examination may be made either to determine the potassium hydrogen tartrate present or the total tartaric acid that will be yielded by the sample.

Estimation of Potassium Hydrogen Tartrate.—Oulman's method (Lunge, Chem. Techn. Unters. Meth., Vol. 3):

0.376 grm. of the finely-powdered sample are put into a 1000 c.c. flask with 750 c.c. of water, boiled for, at most, 5 minutes, made up to the mark, cooled, again made up to the mark, mixed and 500 c.c. of filtrate collected through a dry filter. This filtrate is evaporated to dryness in a porcelain basin on the water-bath. While the dry mass is still warm, it is moistened with 5 c.c. of water cooled. and 100 c.c. of alcohol added, the mixture thoroughly stirred and allowed to stand for 30 minutes. The alcohol is then decanted through a dry filter, and the last portion drawn through with the pump. Any acid potassium tartrate on the filter is washed back into the evaporating basin with boiling water, the solution diluted with water to make 100 c.c. and titrated with N/5 alkali. 0.2 c.c. should be added to the titration figure for correction.

Total Tartaric Acid.—

The following process for analysis of tartar is designated "Goldenberg 1907" (*Zeit.*, *anal. Chem.*, 1908, 47, 57), and was approved at the 7th *International Congress of Applied Chemistry*, London, 1909. It is now in general use.

A weighed amount of the sample (6 grm. if the tartaric acid yield is likely to be above 45%; 12 grm. if below that amount) is treated with 18 c.c. of hydrochloric acid (sp. gr. 1.1) for 10 minutes. The whole mass is then rinsed into a 200 c.c. measuring flask, made up to the mark with distilled water, shaken well and filtered through a dry filter into a dry flask. 10 c.c. of potassium carbonate solution (66 grm. of absolute carbonate in 100 c.c.) are placed in a 300 c.c. beaker and 100 c.c. of the filtered liquid added. The capacity of the pipette by which this volume is measured must correspond exactly with that of the flask. The mixture is brought to boiling and kept at that point for 20 minutes, until the calcium carbonate has separated

in crystalline form. The liquid and precipitate are washed into a 200 c.c. flask, cooled, made up to the mark, shaken well and filtered through a dry filter. A volume of 100 c.c. of the filtrate is placed in a porcelain basin or Jena flask and evaporated on the hot plate to 15 c.c. and, while the liquid is hot, 3.5 c.c. of glacial acetic acid are added gradually and with constant stirring which is continued for five minutes after all the acid has been added. After 10 minutes' standing, 10 c.c. of alcohol (95%) are added and the liquid stirred for another 5 minutes, and after standing for another 10 minutes the liquid is filtered by the aid of a pump and washed with alcohol until the washings are no longer acid. (See below.) The filter and precipitate are transferred by the aid of 200 c.c. of hot water into a porcelain basin, the liquid brought to boiling and titrated with N/5 alkali and neutral litmus-paper. The alkali must be standardised with the same paper, using pure potassum hydrogen tartrate. As the volume of undissolved matter is disregarded in making up the dilu- tions, an allowance must be made. It is agreed that for samples yielding less than 45% of acid, 0.8 should be deducted; for samples yielding from 45% to 60%, 0.3 should be deducted; for those yield- ing 60% to 70%, 0.2 should be deducted; for yields over 70% no deduction is made.

To control the washings it is advised that 30 c.c. of the alcohol that is to be used should be titrated with standard alkali, using phenol- phthalein, and that the washing should be continued until 30 c.c. of the filtrate require the same amount of standard alkali (with phenol- phthalein) to give the color that was produced in the test of the original 30 c.c..

Porcelain dishes marked with a ring at the volume of 15 c.c. can be obtained.

Warrington's Method for Wine Lees.—Place 8 grm. of the sample in a beaker, moisten with water and heat on water-bath about 5 minutes. Add 2 grm. of potassium oxalate and heat the mixture 15 minutes on water-bath. While hot *almost* exactly neutralise with potassium hydroxide solution (3.5% solution), taking care not to neutralise completely, and avoiding an excess of alkali. The quantity of alkali used is about 0.5% short of that required for complete neutralisation, as ascertained by a separate experiment (see below). After neutralisation in this way, heat on water-bath about 30 minutes and filter, preferably on filter pump, using porcelain

plate 2.5 cm. diameter. (For difficulties experienced with slow filtering material see Grosjean, *Trans.*, 1879.) Wash with 10 lots of water, using 3 c.c. at a time. This should be sufficient, and the filtrate should have a volume of about 50 c.c. *Make to this volume* either by addition of water or by evaporation. Add 5 grm. potassium chloride and 2.5 grm. citric acid, stir well *continuously* during 10 minutes and let stand. Filter off the potassium hydrogen tartrate on pump, wash with a 10% solution of potassium chloride saturated with potassium hydrogen tartrate, of which the acidity has been ascertained by N/10 alkali. When the acidity of the washings is the same as that of the washing solution, dissolve the precipitate in hot water and titrate with N/10 potassium hydroxide.

For Tartars.—Use 3 grm. and proceed as above.

Preliminary Determination of Acidity.—Extract exactly 3 grm. of the sample by boiling with water, decanting, again boiling and again decanting. The residue is transferred to filter-paper and thoroughly washed until the washings are no longer acid, titrated with N/10 alkali or with 3.5% potassium hydroxide solution, using neutral litmus-paper.

The following special precautions applicable to the above processes are taken from Rasch's book (Die Fabrikation der Weinsäure):

The sample must be ground very fine. The alcohol and water must be neutral to the indicators employed. The potassium carbonate should be pure, especially free from iron and aluminum. The procedures must be at ordinary temperature unless otherwise directed. The evaporation of the solution containing potassium carbonate must not be carried too far and the treatment with acetic acid must be while the liquid is hot. These conditions are necessary to secure crystalline precipitates. The acetic acid must not be below 98%.

The washing with alcohol must be carefully carried out. It is best to stir the precipitate with the stream from the jet of the washbottle, and then wash the funnel margin above the filter. Usually it will be sufficient to fill the filter in this manner 3/4 full five successive times.

The standard potassium hydroxide must be free from carbonate and be accurately titrated with pure potassium hydrogen tartrate, using exactly the same kind of litmus-paper that is used in the assay.

Calcium Tartrate Assay.—The following method was adopted

at the Seventh International Congress of Applied Chemistry (London, 1909)[1].

6 grm. are always to be taken and the potassium carbonate solution is *added to the* 100 c.c. of the hydrochloric acid solution, drop by drop by means of a pipette, at such rate as to require in all about five minutes. The mixture is boiled for 20 minutes longer as directed above. The modified procedure is to avoid the occlusion of calcium tartrate in the calcium carbonate.

For the estimation of calcium carbonate in calcium tartrate, the carbon dioxide that the sample will yield must be weighed directly.

Commercial Cream of Tartar.—Allen suggested (*J. Soc. Chem. Ind.*, 1896, **15**, 681) the following methods:

1. Dissolve 1.881 grm. of the sample, free from moisture, in hot water and titrate with N/10 alkali, phenolphthalein being used as an indicator. In the absence of acid potassium sulphate and tartaric acid, each c.c. of alkali represents 1% of acid potassium tartrate.

2. Ignite 1.881 grm. for 10 minutes, boil with water, filter and wash the residue.

a. Titrate the filtrate with N/10 hydrochloric acid and methylorange. With pure tartar, the quantity of acid used will equal that consumed in the previous titration with alkali. Each c.c. of the deficiency of acid is equivalent to 0.36% of calcium sulphate, or 0.72% of potassium hydrogen sulphate. Any excess of acid added points to the presence of potassium tartrate, each c.c. representing 0.6% thereof. If the titrated liquid be treated with barium chloride, the barium sulphate will be a measure of the calcium sulphate or potassium sulphate present.

b. The carbonaceous residue is ignited, dissolved in 20 c.c. of N/10 acid, filtered from any insoluble residue, and the filtrate titrated with N/10 alkali. Each c.c. corresponds to 0.50% of calcium tartrate or 0.36% of calcium sulphate (anhydrous).

The following processes, described by Rasch, are included in Lunge's Chemische-Technische Unters. Methoden, being for analysis required in the routine of tartar works. The potassium carbonate solution directed contains 5 grm. of the pure salt in 100 c.c. of solution. Phenolphthalein is used as indicator and N/10 potassium hydroxide for titration.

[1] I am indebted to Mr. W. A. Davis for a special communication advising me of this process.—H. I..

Tartaric Liquors.—10 c.c. are boiled with 40 c.c. of the potassium carbonate solution for a short time, made up to 200 c.c., filtered through a dry filter, 10 c.c. of the filtrate mixed with 3 c.c. of glacial acetic acid and 100 c.c. of alcohol and the precipitate titrated. The number of c.c. used multiplied by 30 will give grm. of tartaric acid yield per 1000 c.c. of liquor.

Old Mother-liquors.—10 c.c. of this are mixed with 60 c.c. of potassium carbonate solution, boiled, cooled, made up to 200 c.c., filtered through a dry filter, 20 c.c. of the filtrate mixed with 5 c.c. of glacial acetic acid and 100 c.c. of alcohol and the precipitate titrated. The c.c. used multiplied by 15 will give grm. of tartaric acid per 1000 c.c. of liquor.

Residuums.—300 grm. are dissolved in a porcelain basin with 25 c.c. of hydrochloric acid (sp. gr. 1.1) and 500 c.c. of water, the mixture being heated to boiling with constant stirring. A portion of the liquid is filtered, 50 c.c. of the filtrate mixed with 5 c.c. of glacial acetic acid and 130 c.c. of alcohol. The precipitate is titrated. Each 5 c.c. of this required will be approximately equal to 0.1% of tartaric acid in the material.

Mother-liquor from Calcium Tartrate Precipitates.—200 c.c. are evaporated to 50 c.c., boiled for a few minutes with 10 c.c. of the potassium carbonate solution, made up to 100 c.c., filtered through a dry filter, 60 c.c. of the filtrate mixed in a measuring flask with 10 c.c. of hydrochloric acid (sp. gr. 1.1) and alcohol added to make a volume of 180 c.c. The mixture is shaken, filtered promptly through a dry filter, and the following are added in succession to 150 c.c. of the filtrate. Ten c.c. potassium carbonate solution, 5 c.c. glacial acetic acid, and 100 c.c. of alcohol. The mixture is well shaken and allowed to stand for twenty-four hours.

The precipitate is titrated. Each 10 c.c. used will be equivalent to 1.5 grm. tartaric acid in 1000 c.c. of the liquor.

Free Sulphuric Acid in Liquors.—20 c.c. of the liquor are made up to 200 c.c. with alcohol, allowed to stand overnight, filtered through a dry filter, 100 c.c. of the filtrate cleared of alcohol and precipitated with barium chloride as usual.

Detection of Lead.—The following process is from a description furnished by W. A. Davis, but this has been modified by a further communication, for abstract of which, see Appendix, page 569

10 grm. of tartaric acid are dissolved in about 20 c.c. of distilled water, the solution filtered if necessary and placed in a tall, narrow cylinder of colourless glass marked at 100 c.c. Solution of hydrogen sulphide (made by passing the gas through water for at least two hours before using) is added in amount sufficient to make 100 c.c. and after 10 minutes the color of the solution is noted. If no colour is produced lead is absent, or at least below 0.0005%. This is the case with the best product.

A slight bluish turbidity represents about 0.00075 %.

A decided blue-yellow or gray represents 0.001 %.

A brown tint may be due to either iron or lead, but the latter is usually distinguished by the blacker tint seen when the liquid is held against a white background.

10 grm. of cream of tartar are heated with about 50 c.c. of water, and ammonia added until all the potassium hydrogen tartrate is dissolved. It the solution is coloured it must be treated with purified animal charcoal and filtered. The liquid is diluted to 100 c.c., as above noted, and 3 drops of ammonium sulphide added. If the reagent is yellow, allowance must be made for this tint.

If no colouration is produced by the reagent, lead is below 0.0005%.

A slight brownish-yellow shows about 0.00075%.

A clear brown tint, about straw coloured, shows about 0.001%.

Copper is shown by the blue tint imparted to the ammonia solution before the sulphide is added; iron shows a dark green precipitate or a green tint. These tints mask the lead reaction and in such cases a few drops of potassium cyanide solution must be added to the alkaline solution before filtering, and the above procedure followed.

For the separation of ordinary tartaric acid, mesotartaric acid, and the racemic association of two active forms, Hollemann (*Rec. Trav. Chim.*, 1898, **17**, 66) devised the following method: The aqueous solution of the free acid is evaporated in the water-bath until crystallisation begins, and the liquid is allowed to stand in a cool place for 24 hours. Racemic acid separates and the crystals may be carefully drained, dried, and weighed. The mother liquor is diluted to 20 c.c.; one-half of this exactly neutralised by potassium hydroxide, the other half added, and the mixture allowed to stand overnight. Potassium hydrogen tartrate separates quantitively, and can be collected, dried, and weighed. The filtrate is treated with ammonia, then slightly

acidified with acetic acid, boiled, and calcium chloride solution added. The calcium mesotartrate is thrown down.

Detection of Yeasts in Lees.—Rasch states (*Lunge, Chem. Tech. Unters. Meth.*, vol. 3) that it is sometimes advisable to ascertain if lees have an excessive amount of yeast cells, and recommends the following: 40 grm. of the sample are stirred with some water in a 400 c.c. beaker, 50 c.c. of 10% calcium chloride solution added, the solution neutralised accurately with milk of lime, the beaker filled with water, and the mixture kept at 35° for 24 hours. Good, well-dried lees will not show appreciable fermentation.

Tartrates.—Tartaric acid contains 4 atoms of replaceable hydrogen but only 2 of these are in the true acid-forming position, hence the acid is bibasic and with members of the potassium group forms 2 series of salts, tartrates and hydrogen tartrates, the latter being often erroneously called "bitartrates." Few of the salts are soluble in water, and all are insoluble in alcohol. The salts of the members of the potassium group unite readily with those of some of the other groups to form double tartrates which are not decomposed on adding strong hydroxides. In this way, the addition of sodium potassium tartrate - to copper sulphate solution will prevent entirely the precipitation of copper hydroxide on adding sodium hydroxide. This mixture is known as Fehling's solution. The analysis of these double tartrates is described on page 538.

Potassium Tartrates.

The most important of these salts is the potassium hydrogen tartrate, often erroneously called bitartrate. This is the principal constituent of tartar, argol, and wine-lees, and is of importance in the pure state as a source of tartaric acid and as a form for the determination of that body.

Pure potassium hydrogen tartrate may be conveniently prepared by dividing a solution of tartaric acid into two equal parts, neutralising one portion with potassium carbonate, and adding the other. The product may be purified by recrystallisation from hot water.

It forms colourless crystals, is soluble in 240 parts of water at 10°, 180 at 20°, and in about 15 parts of boiling water. In alcohol it is much less soluble. It requires (at 15°) 400 parts of a liquid containing 10.5% of alcohol, and for 50% alcohol about 2,000 parts for solution. In still stronger spirit it is practically insoluble. The presence of glucose does not affect its solubility in water or weak alcohol; but

some salts and acids have great influence. This shown by the following table by Warington, in which the effect of water containing equivalent quantities of acids is given. For comparison with them, experiments were also made with solutions containing equivalent amounts of acetic and citric acids neutralised by potassium hydroxide. All the experiments were made at 14°:

Solvent	Grm. of acid or salt in 100 c.c. of Solvent	Grm. of tartrate dissolved by 100 c.c. of solvent
Water..	....	.422
Acetic acid..................................	.8106	.422
Tartaric acid................................	1.0331	.322
Citric acid..................................	.8448	.546
Sulphuric acid..............................	.6853	1.701
Hydrochloric acid...........................	.5037	1.949
Nitric acid..................................	.8445	1.969
Potassium acetate...........................	1.3875	.744
Potassium citrate...........................	1.3966	.843

These results are of importance in the estimation of tartaric acid as potassium hydrogen tartrate. Mineral acids should not be present nor any large excess of potassium acetate or citrate. On the other hand, solutions of potassium sulphate, nitrate and, especially, chloride have very little solvent action on the precipitated tartrate. Thus the solubility of the potassium hydrogen tartrate at 12° is 1 part in 3213 of a 5% solution of potassium chloride, and only 1 in 4401 of a 10% solution of the same salt.

Potassium hydrogen tartrate dissolves many oxides, forming double tartrates; tartar emetic is a compound of this character.

Cream of tartar consists chiefly of potassium hydrogen tartrate. Its composition and the mode of assaying it are considered on page 548.

When potassium hydrogen tartrate is treated with solution of potassium carbonate or hydroxide until the liquid ceases to redden litmus-paper, there results:

Potassium tartrate; neutral potassium tartrate. This forms colourless crystals freely soluble. When its solution is treated with an acid, the hydrogen tartrate is precipitated. ·

Potassium sodium tartrate, Rochelle salt is produced by neutralising cream of tartar with sodium hydroxide or sodium carbonate.

It forms large crystals, containing 4 mol. of water, and is very readily soluble. Addition of acetic acid precipitates crystalline potassium hydrogen tartrate. This reaction distinguishes it from the sodium tartrate.

Seidlitz powders contain potassium sodium tartrate. Sometimes the tartrate is largely, and occasionally entirely, replaced by sodium hydrogen carbonate. Such a preparation would be strongly alkaline and notably different from Seidlitz powder. On the other hand, if the acid is in excess, the powder is apt to produce a turbid solution with water, owing to formation of potassium hydrogen tartrate.

In examining Seidlitz powders, the absence of notable proportions of sulphates should be proved, as a substitution of potassium hydrogen sulphate for tartaric acid is not unlikely. Some powders receive an addition of magnesium sulphate, or a minute quantity (1/100 grain) of tartar emetic, while others are flavoured with lemon or ginger, and sweetened with sugar. Potassium chlorate is a constituent of some proprietory remedies of the nature of Seidlitz powders.

Potassium Ferric Tartrate.—Prepared by adding precipitated ferric hydroxide to acid potassium tartrate and treating with cold water. It constitutes the *ferrum tartaratum* of pharmacy. The solution acidulated with hydrochloric acid should give a copious blue precipitate with the ferrocyanides but none with the ferricyanides. It should yield 30% of Fe_2O_3, as estimated from the weight of the ash insoluble in water.

Potassium Antimonyl Tartrate.—Tartarised antimony; tartar emetic. This is prepared by mixing antimonious oxide with potassium hydrogen tartrate, and subsequently adding water, boiling, filtering and crystallising. Cold water dissolves 7%, and boiling water 53% of the salt; the solution has an acid reaction. *Antimonial wine* is a solution of tartar emetic in wine.

Tartar emetic is now extensively employed for fixing certain coal-tar colours on cotton, its value for this purpose depending on the content of antimony. It is frequently largely adulterated, the percentage of antimony being sometimes scarcely one-half of that present in the pure substance.

The antimony may be conveniently estimated volumetrically, in a manner described by W. B. Hart (*J. Soc. Chem. Ind.*, 1884, 3, 294). The sample is dissolved in water and acid sodium carbonate added to the solution. Excess of a standard solution of calcium

hypochlorite is then added. The excess is found by titrating back with a N/10 solution of sodium arsenite until a drop of the liquid ceases to give a blue with potassium iodide and starch. The strength of the hypochlorite solution is found by taking a measure equal to that added to the antimony solution and titrating with arsenite as before. 1 c.c. of a solution prepared with 4.95 grm. of pure arsenous oxide per litre has the same reducing power as 0.0060 grm. of antimony or 0.0072 of antimonous oxide.

Potassium antimonyl oxalate, has been used as an adulterant of, and substitute for, tartar-emetic. It is readily soluble, does not blacken on ignition or on heating with sulphuric acid, and gives a white precipitate on adding calcium chloride to the solution previously acidified with acetic acid. The salt yields only 23.7% of antimonous oxide.

Ammonium tartrates closely resemble the corresponding potassium salts, but are wholly volatile on ignition.

Calcium tartrate, is a natural constituent of tartar from wine, the proportion contained being much increased if the wine has been "plastered." It also constitutes the greater part of the residue obtained on treating commercial tartars with hot water. Calcium tartrate is precipitated as a crystalline powder containing 4 mol. of water by adding excess of calcium chloride to a solution of a tartrate. It is soluble in 6265 parts of water at 15° and in 352 parts of boiling water. Strong acids and potassium hydrogen tartrate dissolve it readily; and hence it is frequently present in notable quantity even in purified tartars. These solutions are precipitated by ammonium hydroxide, either immediately or after some time. Calcium tartrate is soluble in ammonium chloride and in cold alkali, the latter solution being reprecipitated on boiling. By digestion with a hot neutral solution of copper chloride it is converted into insoluble copper tartrate. This reaction distinguishes it from calcium *citrate*, but the reaction fails with mixtures containing a large proportion of citrate. The tartrate differs from the *racemate* and *oxalate* by its solubility in acetic acid. (For assay of crude calcium tartrate see p. 547.)

Calcium racemate, is even less soluble in water than calcium tartrate, and is precipitated in fine needles on adding calcium sulphate to a soluble racemate or even to a solution of free racemic acid. Calcium racemate resembles the oxalate in being insoluble in acetic acid. It dissolves in hydrochloric acid to form a solution which is at once pre-

cipitated on adding ammonium hydroxide, whilst the tartrate is not precipitated for some time.

Citric Acid.

Citric acid occurs in a free state in the juices of many plants of the genus of *Citrus* (order, *Aurantiaceæ*), and also in the gooseberry, cranberry, currant, tamarind and many other fruits. The lemon, lime and bergamot are the fruits from which it is extracted. It has also been manufactured from unripe gooseberries, which yield about 1% of their weight of citric acid, besides containing malic acid. Good lemons yield about 5.5 % of crystallised citric acid. Calcium and potassium citrates are also widely distributed in the vegetable kingdom.

Citric acid is prepared from lime, lemon or bergamot juice, by neutralising the liquid with calcium carbonate, decomposing the resultant calcium citrate by an equivalent amount of sulphuric acid, and evaporating the liquid to the crystallising point.

Citric acid usually occurs as a crystalline powder or in transparent colourless prisms. In the trade, the crystals are assumed to have the composition $C_6H_8O_7 + H_2O$.

Crystallised citric acid begins to lose water at 75°, becomes anhydrous at 135°, fuses at 153°, and at about 175° decomposes into water and aconitic acid, $C_6H_6O_6$.

Citric acid has a strong acid taste, is soluble in about half its weight of water at 25° and 0.4 part of boiling water, in 1.5 parts of strong alcohol at 25° and 1.4 of boiling alcohol and in 18 parts of ether. The solution has no optical activity. Aqueous solutions readily mold.

Citric acid is very soluble in dilute and absolute alcohol, but is nearly insoluble in ether, chloroform, benzene or petroleum spirit.

Detection and Estimation of Citric Acid and Citrates.— When 5 grm. of citric acid are heated with 30 c.c. of ammonium hydroxide for 6 hours in a sealed tube at a temperature of 120°, a yellow colouration is observed and small crystals are formed. If the cooled liquid be poured into an evaporating basin, it becomes blue in the course of some hours, the colour becoming more intense on standing, and in a few days turning to green, and ultimately disappearing. The change of colour goes on more slowly in the dark. Heating the liquid on the water-bath hastens the production of the colour. Malic, tartaric, and oxalic acids do not interfere, even when present in large excess, but

itaconic acid must be absent. It is said that 0.01 grm. of citric acid can be detected by this process (*Zeits. Anal. Chem.*, 1878, **17**, 73).

Calcium citrate is very sparingly soluble, and less soluble in hot water than in cold. Hence, addition of excess of lime-water to a solution of citric acid produces but a slight precipitate in the cold, but a somewhat more considerable precipitate of calcium citrate is obtained on boiling, the deposit redissolving as the solution cools.

Precipitation as calcium citrate may be employed for the estimation of citric acid, and serves to separate citrates from *malates*, *acetates*, *formates* and *butyrates*; but the precipitate may contain calcium tartrate, oxalate or racemate.

Citric acid may be roughly separated from tartaric acid by digesting the mixed calcium salts with a hot and perfectly neutral solution of copper chloride, when soluble copper citrate is formed and an insoluble tartrate remains. In the case of mixed tartrates and citrates which can be converted into the calcium salts by precipitation with calcium chloride or nitrate in perfectly neutral boiling solution, this method of separation is occasionally convenient for qualitative purposes, but it is greatly inferior to the precipitation of the tartaric acid as potassium hydrogen tartrate, and fails wholly if the proportion of tartrate is small.

From *tartaric acid*, citric acid is best separated by the method described on page 558. In the filtrate from the precipitate of potassium hydrogen tartrate the citric acid may be determined by boiling off the lacohol, exactly neutralising with sodium hydroxide, and proceeding as directed on page 561, or by precipitation with barium acetate or lead acetate. If the acids do not exist in the free state, the solution must be prepared as directed under Tartaric Acid.

From *oxalic acid* citric acid is separated by neutralising the solution with sodium hydroxide, acidifying with acetic acid and adding calcium sulphate or chloride. After filtering from the precipitated calcium oxalate, the citric acid may be thrown down by adding lime-water and boiling.

If moderately pure, citric acid may sometimes be conveniently converted into barium citrate by precipitating the neutralised solution with barium acetate and adding 2 volumes of 95% alcohol. After 24 hours, the precipitate is filtered off, washed with alcohol of 63%, ignited, moistened with sulphuric acid, again ignited, and the weight multiplied by 0.601. Alkaline acetates do not interfere, so that the

method is applicable to liquids from which the tartaric acid has been separated as potassium hydrogen tartrate.

In the absence of other acids, citric acid may be titrated with standard alkali, neutral litmus-paper being used. The alkali should be set against pure citric acid.

For the estimation of citric acid in presence of heavy metals, the latter should be first removed by hydrogen sulphide or sodium sulphide and the filtered liquid rendered neutral and precipitated with excess of lead acetate. The unfiltered liquid is mixed with an equal volume of alcohol, filtered, the precipitate washed with proof spirit and treated with ammonium hydroxide. The filtrate may contain citric and tartaric acids, but will be free from sulphates, phosphates and oxalates. When unmixed with other lead salts, lead citrate may be suspended in water, decomposed by hydrogen sulphide, the liquid filtered, well boiled and the citric acid in the solution titrated with alkali.

Full descriptions of the methods of determining citric acid in *juices* and *citric acid liquors*, will be found in subsequent paragraphs.

Commercial citric acid frequently contains small quantities of *calcium salts*, due to imperfect manufacture, and traces of *iron, lead* and *copper* are also met with—these last being derived from the vessels used for the crystallisation and evaporation of the acid liquids.

The presence of all these impurities is indicated by igniting 5 or 10 grms of the sample in a porcelain crucible. The ash usually ranges from 0.05 to 0.25%. When the ash does not exceed the latter amount, it is rarely of importance to examine it further, except for poisonous metals.

For the detection of lead the procedure is the same as with tartaric acid (p. 550). See also appendix, page 569;

A colourless solution shows below	0.0003 %.
Faint blue " " "	0.00075%.
Decided blue yellow " "	0.001 %.

The presence of poisonous metals in citric acid is accidental, and the proportion present is usually small (1 part in 10,000); but as lead and copper are occasionally present in dangerous amount, it is necessary to take every precaution to avoid their introduction.

If samples of citric acid contain *sulphuric acid*, they will be deliquescent. Sulphuric acid and sulphates may be detected and determined by acidifying rather strongly with hydrochloric acid and

adding barium chloride. 233 parts of the precipitate correspond to 98 of sulphuric acid.

Formerly citric acid was liable to adulteration with tartaric acid. If present, tartaric acid may be conveniently detected by the charring which occurs on heating the sample with concentrated sulphuric acid, as described on page 538. When the proportion of tartaric acid in admixture with the citric acid is not too small, it may be detected by the dark mixture produced, within 5 minutes, when 1 grm. of the sample is dissolved in 10 c.c. of a cold saturated solution of potassium dichromate.

For the detection of tartaric acid in citric acid, Vulpius dissolves 0.5 grm. of the sample in 10 c.c. of distilled water, and adds 5 drops of the solution, drop by drop, to 15 c.c. of lime-water. If the citric acid contain mere traces of tartaric acid, a distinct turbidity will be produced in a few moments, which increases on adding more of the acid solution and stirring. In this manner 1% of tartaric acid may be detected.

If present in admixture with citric acid, *tartaric acid* is best estimated by the methods on pp. 545 to 548.

Citric Acid Liquors.—This term is applied to the liquors resulting in citric acid works from the treatment of the calcium citrate with sulphuric acid. The assay is limited to the estimation of citric and sulphuric acids. For this purpose the total acidity may be determined by titration with standard alkali and phenolphthalein, and the sulphuric acid then determined. By subtracting the acidity due to the latter from the total found by titration, that due to the citric acid alone is ascertained. The free sulphuric acid is ascertained by treating 10 or 20 c.c. of the liquor with 5 times its volume of strong alcohol. After twelve hours a portion of the clear liquor is treated with more alcohol, and, if opalescence result, the whole is treated in the same way. The liquid is ultimately filtered, the precipitated sulphates washed with spirit and the filtrate precipitated with an alcoholic solution of calcium chloride. The precipitated calcium sulphate is allowed to settle completely, the supernatant liquor poured off, and the precipitate and small quantity of remaining liquor *gently warmed*. The alcohol is gradually displaced by cautious additions of small quantities of water, and, when the precipitate has become crystalline, alcohol is added, and the precipitate collected on a filter, washed with alcohol, ignited, and weighed as calcium sulphate. The weight multiplied by 0.7206 gives the sulphuric acid (H_2SO_4) in the liquor taken.

Another method, which agrees well with the above is to neutralise exactly a known measure of the citric liquor with pure sodium hydroxide evaporate to dryness, and ignite gently in platinum. The ash is wholly dissolved in a known quantity of standard acid, and the excess of acid ascertained by titration with alkali. (In presence of iron or aluminum, sodium tartrate or sodium potassium tartrate should be added before titration.) The acid neutralised by the ash is equivalent to the organic acid contained in the liquor used.

In old liquor, the citric acid should be precipitated as calcium salt, as other organic acids will be present in considerable amount. For this purpose the liquor is treated exactly as directed for juice.

Lemon Juice; Bergamot Juice; Lime Juice.—These juices contain citric acid; acids other than citric; citrates; salts of organic acids other than citric; salts of inorganic acids; and albuminous, mucilaginous, saccharine and indifferent bodies. Alcohol is frequently added as a preservative, and mineral acids are not uncommonly employed as adulterants. Verjuice has also been used for the purpose.

J. Macagno finds that the alcoholic fermentation which takes place when freshly expressed lemon-juice is kept does not diminish the amount of citric acid present, but that this is succeeded by another fermentation which diminishes the citric acid and other organic acids (chiefly acetic and propionic) increase. Similarly, juice expressed from rotten fruit contains acids other than citric, sometimes to the extent of 10%c.

Citric acid juices lose some of their acidity by concentration. Warington observed a loss of 3.5% of the total free acid on concentrating English-pressed juice to 1/6 of its original bulk. The loss is due, at least in part, to the presence of volatile organic acids, which, of course, exist in much smaller amount in concentrated juice. Warington found 1.25% of the total acidity of concentrated juice to be due to volatile acids. Among the latter were recognised formic, acetic and probably propionic acids.

The following table, compiled from Warington's data, shows the sp. gr. acid, and combined organic acid (the last two expressed in terms of crystallised citric acid) of the various citric juices commonly met with in commerce.

	Specific gravity	Acid, oz. per gallon	Combined organic acid oz. per gallon
Lime juice:			
Raw Sicilian...................	....	6–9	0.85
Raw English...................	1.04 –1.05	11–13	0.3
Concentrated.................	1.20 –1.25	56–72	6–8
Bergamot juice:			
Concentrated.................	1.22 –1.25	47–55	7–8
Lemon juice:			
Raw.........................	1.035–1.040	10.6–13.5	0.4–0.7
Concentrated.................	1.28 –1.38	82–112	8–6

In the following table, due to Grosjean, are given determinations of the free acid and precipitable organic acid (both calculated as citric acid) in commercial samples of concentrated lemon and other juices:

	Specific gravity	Acid (reckoned as citric acid), oz. per gallon		Proportion of precipitable to 100 of acid
		Acid	Total acid precipitable	
Lemon juice:				
Average of 65 samples	1.241	62.1	61.6	99.2
Sample A....................	1.240	65.8	59.7	90.7
Sample B	1.235	64.9	55.7	85.8
Bergamot juice:				
Highest.....................	1.235	47.9	48.5	101.4
Lowest......................	1.235	52.3	49.9	95.4
Lime juice:				
Sample A....................	1.326	108.3	99.8	92.2
Sample B....................	1.205	59.2	53.9	91.1
Orange juice:				
Sample A....................	1.400	16.8	11.6	69.0
Sample B....................	1.350	11.7	8.0	68.4

From the first of these tables it will be seen that English-pressed juice contains more free and less combined acid than the raw Italian and Sicilian juices. This is probably due to the fact that the finest and ripest fruit is sent to England, while the windfalls and damaged fruit are treated locally.

Concentrated bergamot juice is far less acid than lemon juice, while concentrated lime juice is a thick viscid fluid far exceeding the others both in density and acidity.

The assay of genuine juice is practically confined to the estimation of citric acid and citrates, and for this purpose the following processes are employed:

Specific Gravity.—A special hydrometer is sometimes used. On this "citrometer," 60 degrees correspond to a sp. gr. of 1.240, so that each degree appears to be equal to 0.004 sp. gr. above unity.

The valuation by sp. gr. is open to many frauds. Bergamot juice, which has a high gravity but low acidity, has been mixed with lemon juice, and sea-water has been added to the juice during concentration. Of course, the presence of alcohol materially affects the density, but it may be got rid of by boiling the juice and again taking the sp. gr. after making up the volume to that originally employed.

Estimation of the Acid.—This is effected by titration with $N/2$ sodium hydroxide, neutral litmus-paper being used as an indicator. In the case of concentrated juice, 50 c.c. should be diluted to 500, and 25 c.c. to 30 c.c. of the diluted liquid employed for the titration. With unconcentrated juice, 10 c.c. or 20 c.c. may be measured out at once. In either case, the alkali is added in quantity sufficient to neutralise about 80% of the acid present; the liquid is then boiled for a few minutes, and when quite cold the titration is completed. The neutralising power of the alkali should be known in terms of pure citric acid.

Estimation of the Citrate and Other Organic Salts.—This is effected by evaporating to dryness the portion of juice which has been already neutralised for the determination of free acid. The residue left on evaporation is heated gradually, and charred at a low red heat. The ignited mass is treated with water, a known volume of standard sulphuric acid added, the liquid boiled and filtered, and the excess of acid ascertained in the filtrate by standard alkali. The amount of sulphuric acid neutralised by the ash is equivalent to the total organic acid of the sample, for on ignition all the salts of organic acids were converted into the corresponding carbonates. 49 parts of sulphuric acid neutralised = 40 of sodium hydroxide = 70 of $H_3C_6H_5O_7,H_2O$, or 67 of $2H_3C_6H_5O_7,H_2O$.

The result gives the total organic acid of the juice taken, calculated as citric acid. By subtracting the amount of free citric acid, obtained

by titration of the acid juice, the amount of combined citric acid is ascertained.

If the original acid juice is evaporated and ignited, and the combined citric acid calculated from the neutralising power of the ash, the results obtained are too high, owing to the decompositions by the citric acid during evaporation.

Estimation of the Real Citric Acid.—Of the organic acids present in genuine lemon and similar juices, the citric is the only one of importance which forms an approximately insoluble calcium salt. Calcium malate and aconitate are pretty freely soluble, and the same remark applies more strongly to calcium acetate and butyrate produced by the fermentation of citric acid juices. For the determination of the amount of insoluble calcium salt obtainable from a citric juice, R. Warington recommends the following method (*Jour. Chem Soc.*, 1875, **28**, 934):—15 to 20 c.c. of unconcentrated lemon juice, or about 3 c.c. of concentrated juice (previously diluted to facilitate exact measurement) should be exactly neutralised with pure sodium hydroxide. The solution is brought to a bulk of about 50 c.c. and heated to boiling in a salt or glycerol bath, and so much of a solution of calcium chloride added as is known to be rather more than equivalent to the total organic acids present. The whole is boiled for half an hour, and the precipitate then collected and washed with hot water. The filtrate and washings are concentrated to about 10 or 15 c.c., the solution being finally neutralised .with a drop of ammonia if it has become acid. The second precipitate thus obtained is collected on a very small filter, the filtrate being employed to transfer it, and the washing with hot water being reduced as much as possible. In very accurate experiments the concentration should be repeated and any further precipitate collected. The precipitates, with the filters, are then burnt at a low red heat, and the neutralising power of the ash ascertained by treatment with standard hydrochloric acid and alkali. One c.c. of normal acid neutralised corresponds to 0.070 grm. of crystallised citric acid ($C_6H_8O_7,H_2O$). The presence of mineral acids does not interfere; oxalic or tartaric acid would render the results inaccurate. It is desirable to add neutral hydrogen peroxide to the solution of the ash and boil before titrating, otherwise an error may occur from the presence of sulphides.

Official United States Method for Citric Acid in Fruit Juices. (*Bull.* 107, *Bur. of Chem.*, *United States Depart. Agriculture*, page 81).—

50 c.c. of the liquid are evaporated on the water-bath to a syrup, alcohol (95%) added slowly and with constant stirring until no more precipitation occurs. About 80 c.c. will generally be required. The precipitate is collected on a filter and washed with alcohol (95%), the alcohol driven out of the filtrate by evaporation, the residue is taken up with a little water, transferred to a cylinder and made up to 10 c.c. 5 c.c. of this solution are mixed with 0.5 c.c. of glacial acetic acid, and then, drop by drop, a solution of lead acetate added. A precipitate which dissolves when the liquid is heated, and reappears when it is cooled, indicates citric acid. If this is present, the liquid is heated to boiling, filtered hot, the filter washed with boiling water and the filtrates allowed to cool. The lead citrate that separates can be collected on a filter, washed with weak alcohol, dried and weighed. If tartaric acid is present its interference may be prevented by adding N/10 alkali in sufficient amount to neutralise it before adding the alcohol in the beginning of the operation. Lead citrate multiplied by 0.483 gives citric acid.

A method for the analysis of *calcium citrate* and *lemon juice* has been recently described by L. and J. Gadais (*Bull. Soc. Chim.* [4], 1909, 5, 287): 20 grm. of the calcium citrate are boiled for a few moments with 30 c.c. of water and 20 c.c. of hydrochloric acid (sp. gr. 1.28), cooled, made up to 250 c.c., filtered through a dry filter, and 25 c.c. exactly neutralised with N/1 potassium hydroxide, using phenolphthalein, then treated with 1 c.c. of a saturated solution of calcium chloride, evaporated to 25 c.c., and filtered while very hot. The precipitate is washed 8 times with boiling water, using as little as possible, and dried at 105°. The filtrate is concentrated to 15 c.c., any additional precipitate washed 5 times with boiling water, sparingly as before, and dried at 105°. The filtrate and washings may be concentrated to 15 c.c., and any precipitate treated as with the other two. Finally, an equal volume of alcohol is added to the liquid, and a precipitate is added to the others, after drying. The collected precipitates are burnt apart from the filter, and the residue, calcium carbonate, mixed with 30 c.c. of N/1 hydrochloric acid, and the excess of acid ascertained by means of N/1 potassium hydroxide. The c.c. of acid required to neutralise the residue, multiplied by 0.07 gives the amount of citric acid that would be obtained from the sample. If the sample contains much sulphate, it is advisable to burn the precipitate over an alcohol flame, and to add to the residue 10 c.c. of hydrogen peroxide solution

before adding the acid. (The fact that commercial hydrogen peroxide solution generally contains an appreciable amount of acid is not noted in the report of the process, but must not be overlooked. The peroxide solution should either be exactly neutralized or its acidity ascertained and allowance made.)

For *lemon juice*, 120 c.c. are diluted to 1000 c.c., 25 c.c. of this neutralised with N/1 potassium hydroxide, 20 c.c. of saturated calcium chloride solution added, and the procedure carried out as above.

In English-pressed lemon juice the real citric acid is 99% of the total organic acid, but in the concentrated Sicilian juice it ranges from 88 to 95% of the total. In a sample of concentrated bergamot juice, Warington found the precipitable acid to be about 88% of the total organic acid, but a more usual proportion is 96 to 98%. The method of determining the value of juice by its acidity usually, but not invariably, gives tolerably accurate results in the case of lemon and bergamot juice, but in lime juice the results are commonly in excess of the truth. Of course this statement is only true of genuine juice.

Estimation of alcohol can be effected by the usual methods.

Adulterated lime and lemon juices are not uncommon. The production of precipitates with barium chloride and silver nitrate indicates the presence of *sulphuric* and *hydrochloric acids*, respectively, pure juices containing merely insignificant traces of sulphates and chlorides. Free sulphuric acid may also be determined as in citric acid liquors, and both that and free hydrochloric acid by Hehner's method for the determination of mineral acids in vinegar.

According to F. D. Scribani (*Jour. Chem. Soc.*, 1878, 34, 914), *nitric acid* has occasionally been used for the adulteration of lemon juice. On concentrating such juice the nitric acid decomposes the citric acid, either wholly or partially, with formation of oxalic, acetic, and carbonic acids; so that on neutralising the juice with lime a mixture of calcium salts is obtained. To detect the nitric acid, Scribani adds to the juice an aqueous solution of ferrous chloride, strongly acidulated with pure hydrochloric acid and quite free from ferric salt. The liquid is then boiled for a few minutes and, after cooling, tested with a thiocyanate (sulphocyanide). If the liquid ocontain nitric acid, a more or less deep red colour will be produced, owing to the formation of a ferric salt. This test is said to answer equally well in presence of common salt or sulphuric or tartaric acid. In boiled and dark coloured juices dilulion is necessary before the colour can be observed. A more

satisfactory and direct test for nitric acid would be to boil the juice with metallic copper, when red fumes would be produced if nitric acid were present.

Citrates.—Citric acid forms 3 classes of salts. It has a great tendency to produce stable double citrates, and hence many metallic solutions are not precipitable by alkalies in presence of sufficient citric acid. This fact is often utilised in analysis.

No metallic citrate is wholly insoluble in water. Calcium citrate is one of the least soluble and hence is employed in the estimation of citric acid. General reactions of the citrates are described elsewhere, and the properties of the more important commercial forms are given below.

Lithium Citrate.—As usually prepared, this a white powder, but it may be obtained in crystals with 4 mol. of water. The salt is generally stated to be deliquescent, but this is an error. It should be soluble without residue in 25 parts of cold water.

The pure salt, after being rendered anhydrous by drying at 115°, on ignition leaves 52.9% of lithium carbonate. The residue should be treated with ammonium carbonate, and again ignited very gently, as it is liable to lose carbonic acid. A higher ash than the above indicates impurity or adulteration by (probably) *sodium citrate*, which leaves 61.5% on ignition. 1 grm. of anhydrous lithium citrate leaves on ignition a residue which should neutralise at least 14 c.c. of normal hydrochloric acid. The same amount of sodium citrate (after ignition) would only neutralise 11.25 c.c. of acid. If the resultant solution be evaporated to dryness, lithium chloride may be dissolved out of the residue by a mixture of equal volumes of alcohol and ether, while any potassium or sodium chloride will remain undissolved.

Much of the commercial lithium citrate contains *lithium carbonate*. This gives it an alkaline reaction and increases its ash and saturating capacity. Excess of citric acid gives the salt an acid reaction and reduces the percentage of ash and saturating power. Hence these impurities can be distinguished from sodium citrate, which *raises* the ash and *diminishes* the saturating power of the sample.

Potassium salts may be detected by adding tartaric acid to the concentrated solution of the sample and stirring, when a white crystalline precipitate of acid potassium tartrate will be produced.

Insoluble matters, such as powdered petalite or lepidolite, will be

left undissolved on dissolving the sample in hot water, and *calcium* compounds may be estimated in the solution by adding ammonium oxalate.

Calcium Citrate.—This is a white substance, very sparingly soluble in cold, and still less in hot water. It is produced, in an impure state, by the citric acid manufacturer by boiling the juice with calcium carbonate, and is offered in the market as a convenient source of citric acid. The product consists essentially of citrate mixed with other salts of calcium, and excess of lime or calcium carbonate. In Sicily, dolomitic lime is sometimes used for neutralising the juice, in which case magnesium salts will be present. It is particularly liable to decompose if the percentage of moisture is considerable (more than 10 or 12%), and therefore some samples contain scarcely any real citrate.

For the analytical examination of commercial calcium citrate it is sufficient to estimate the citric acid and the excess of carbonate or lime. For the latter purpose, 5 grm. of the sample should be dissolved in a known quantity of weak standard hydrochloric acid kept gently boiling, and, when the solution is quite cold, the amount of acid neutralised is ascertained by titration with standard alkali in the usual manner. Each c.c. of normal acid neutralised by the sample corresponds to 0.050 grm. of calcium carbonate in the portion taken. To determine the organic acids, 2 grm. of the sample should be ignited, the ash boiled with standard acid, the liquid filtered and titrated with alkali. The acid neutralised by the ash is due to the calcium carbonate existing as such in the sample, *plus* that produced by the ignition of the citrate and other organic salts. By substracting the neutralisation due to the form, the equivalent of the organic acids is found; 1 c.c. of normal acid neutralised being equivalent to 0.070 grm. of monohydrated citric acid. This method gives all the organic acids as citric acid, a result which is misleading in decomposed citrate. In such samples, the real citric acid should be determined by dissolving a known weight in hydrochloric acid, exactly neutralising with sodium hydroaide, and treating the precipitated calcium citrate as described on page 562. Magnesium citrate and citrate prepared with dolomitic lime can be correctly analysed by the titration method; but if precipitation be desired, the sample must be decomposed by boiling with sodium carbonate, the magnesium carbonate filtered off, the filtrate neutralised with hydrochloric acid, and precipitated with calcium chloride.

Ferric citrate, may be obtained by dissolving ferric hydroxide

in citric acid and evaporating the solution in thin layers. It is thus obtained in transparent garnet-red scales, which are permanent in the air. It is insoluble in alcohol, but dissolves slowly in water to form a solution of a faintly ferruginous taste, not precipitated by ammonium hydroxide, but yielding ferric hydroxide on boiling with sodium hydroxide. After drying at 100°, the scales should leave from 29 to 30% of residue on ignition.

Iron ammonium citrate may be made by dissolving precipitated ferric hydroxide in a solution of citric acid and adding ammonia. It occurs in thin, transparent, deep red scales, slightly sweetish and astringent. When heated with potassium hydroxide its solution evolves ammonium hydroxide and deposits ferric hydroxide. The alkaline liquid filtered from the precipitate should not give any crystalline precipitate or streaks of potassium hydrogen *tartrate*, when acidulated with acetic acid and vigorously stirred. Ferric ammonium citrate is readily soluble in water, forming a faintly acid solution, but is almost insoluble in 95% alcohol.

APPENDIX.

The detection of lead is materially influenced by the presence of iron. Freshly precipitated ferric hydroxide will, by adsorption, carry down lead hydroxide. The procedure under such conditions has been recently investigated by J. M. Wilkie (*J. Soc. Chem. Ind.*, 1909, **28**, 637) who finds that the usual method of preventing precipitation of iron by adding potassium cyanide succeeds only when the iron is in the ferrous state. As a result of many experiments, Wilkie gives a special process. He uses sodium sulphide as the final precipitant, but W. A. Davis prefers freshly-made colourless ammonium sulphide, prepared by diluting 2 c.c. of 0.880 ammonia to 10 c.c. and saturating with well-washed hydrogen sulphide. Standard lead solutions may be conveniently made from a strong solution of pure lead (5 grm. in nitric acid, evaporated to remove all but a small amount of the acid and made up to 1000 c.c. This strong solution keeps fairly well. For use portions of it are diluted 100 times and applied in the usual manner for colour comparisons.

Tartaric Acid.—Dissolve 10 grm. of the acid in about 25 c.c. of hot water, cool, add 2 c.c. of N/10 sodium thiosulphate, heat to incipient boiling, cool, add 1 c.c. of 10 per cent. potassium cyanide and then ammonia (0.880) in small portions until the solution smells faintly of the reagent. Boil until the liquid is colourless, pour into a tall cylinder, make up to 100 c.c., and add 2 drops of freshly-prepared colourless ammonium sulphide.

The tint developed is compared with tints produced by solutions made from absolutely lead-free acid to which known amounts of lead have been added, so as to give comparisons at say 5 parts per 1,000,000, 10 per 1,000,000, etc.

Cream of tartar should be tested by dissolving 10 grm. of the sample in hydrochloric acid, adding sodium thiosulphate and proceeding as above directed.

Owing to the wide distribution of lead, care must be taken that all the reagents are free from it.

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